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		PREPARATION OF SUGAR BEET WITH CHANGED SUCROSE

(54) Title: DNA SEQUENCES AND PLASMIDS FOR THE PREPARATION OF SUGAR BEET WITH CHANGED SUCROSE CONCENTRATION

(57) Abstract

DNA sequences and plasmids are described, that by integration in a plant genome of sugar beet change the sucrose concentration, as well as transgenic plants that by introduction of the DNA sequences of the invention causes changes in sugar concentration.

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Title: <u>DNA sequences and plasmids for the preparation of</u>
sugar beet with changed sucrose concentration

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Field of the invention

The present invention relates to DNA sequences and plasmids, containing these DNA sequences, which by integration into the genome of a sugar-beet plant, changes the sugar metabolism of the plant to be changed. The invention also relates to transgenic plants formed with the help of these sequences.

Sucrose is of central importance for the plant and serves many functions. For the long distance transport of 15 photoassimilates and/or energy between various organs in ulants, sucrose is almost exclusively used. The sucrose, which is transported in a specific heterotrophic organ, determines the growth and the development of this organ. Thus it is known, e.g. from EP 442 592, that transgenic 20 plants, in which the transport away of the sucrose from the exporting leaves is inhibited by expression of an apoplastic invertase, shows a strong reduction in the growth of e.g. roots or tubers in the case of potato plants. For tobacco plants, the principal importance of 25 sucrose as the central function for the long distance transport of energy carriers within the plant is described (von Schaewen et al, 1990, EMBO J 9: 3033-3044).

30 Further it is also known from EP 455 316 that DNA sequences present on plasmids, after introduction in a plant genome of a potato plant can affect the starch biosynthesis as well altering the amount and composition of the protein in the potato tubers.

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Whilst it has known that a reduction of the amount of sucrose imported in the heterotrophic organs, such as tubers and seeds, leads to loss of yield, it is not known whether an increase in the amount of sucrose in the photosynthetically active parts of the plant, mainly the leaves, leads to a better supply of heterotrophic organs and thus to an increase in yield.

Besides sucrose and/or the hexoses, glucose and fructose,
derived from sucrose, have the property of protection of
plants against frost damage at low temperatures. Frost
damage is one of the main limiting factors in agricultural
productivity in the northern hemisphere. Temperatures
below freezing lead to the formation of ice crystals.

Since the growing ice crystals consist of pure water,
water is abstracted from the cells as the temperature
falls.

This dehydration has at least two potential damaging 20 results:

 All dissolved substances within a cell are strongly concentrated and the cell contracts following the loss of water. Highly concentrated salts and organic acids lead to membrane damage.

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With rehydration from dew, the previously contacted cells reexpand. The cell membrane also expands again. The volume expansion puts a heavy mechanical load on the membrane.

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It is thus clear that a freezing/dew cycle can lead to severe membrane damage of the cells and thus to damage to the plant.

35 It thus appears worth trying to hinder the freezing. One

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possible strategy is the increased formation of osmotically active substances in the cytosol of plant cells. This should lead to a lowering of the freezing point. Osmotically active substances include sucrose and/or the two hexoses derived from sucrose.

The increased formation of sucrose and/or the two hexoses at low temperatures is desirable in the growing plant. Another situation can exist in the harvested parts of a plant, especially in storage.

In relation to the economic aspects, sucrose thus possesses two especially important functions:

- as the transport form for the distant transport of photoassimilates,
- 2 as an osmotically active substance with the desirable activity of lowering the freezing point in intact, growing plants.
- The biosynthesis pathways for the formation of sucrose, either from the primary photosynthesis products (in the leaf) or by breakdown of starch (in the storage organs e.g. of potatoes), are known.
- It is however, not known how and in what way changes of the carbohydrate concentration in sugar beet can be achieved since it is not possible to use even very similar genes such as for example genes that code for a sucrose synthase, ADP-glucose pyrophosphorylase or sucrose phosphate synthase of the potato with satisfactory success for the preparation of sugar beet with changed sucrose concentration. An exact analysis and determination of the DNA sequences or sequence fragments for the sugar beet is thus required.

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To change the sugar concentration in sugar beet, DNA sequences are now provided which code for the small and large subunit of the ADP glucose pyrophosphorylase, the sucrose synthase and the sucrose phosphate synthase of sugar beet (Seq. ID No 1-4).

These DNA sequences can be introduced into plasmids and in this way combined with steering elements for expression in eukaryotic cells. Such steering elements are on the one hand transcription promoters and on the other hand transcription terminators. Each plasmid comprises:

- a) a suitable promoter which ensures that the coding sequence meets a suitable time point or in the specified developments in the transgenic plant or in determined genes of transgenic plants;
 - b) at least a coding sequence for sugar beet that i) is so coupled to the promoter that the formation of an RNA is allowed which is into a protein, whereby the protein demonstrates an enzymatic activity which leads to a change of the sucrose concentration in the plant, or
 - ii) which is so coupled to the promoter that the coding part is read, which leads to the formation of a so-called anti-sense RNA which under-expresses the formation of the protein coded from an endogenous gene in the plant, that is involved in the sucrose biosynthesis; and
 - c) A non-coding termination sequence that contains the signal for the termination and polyadenylation of the transcript.

The coding sequences named under b) are the sequences that code for the large and small subunit of the ADP glucose pyrophosphorylase, for the sucrose phosphate synthase and for the sucrose synthase of sugar beet.

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The large subunit of the ADP-glucose-pyrophosphorylase has the following nucleotide sequence (Seq. ID No. 1):

CAA	\AGAI	AAA 2	ACTT	CCA	T T	CTAC	PTCT	r TG	CACAI	ATAT	AAT"	rtcc	CAC		0050
CAA	rrr	CT ?	KAAT1	ATTT(CT C	ACTT	rcat?	r tai	ATCAC	STTT	TCAC	GCAA	CAT		0100
TCT	ATAC	CTC (SACAI	ACCCI	AC T	TCTC	STTC	r cc	CAAG	ATTC	CAA	ACCT	CTG		0150
AAGCT ATG GAT GCA AGT GCA GCC ATA AAT GTC AAT GCC CAT 024														0200	
AAGCT ATG GAT GCA AGT GCA GCA GCC ATA AAT GTC AAT GCC CAT Met Asp Ala Ser Ala Ala Ile Asn Val Asn Ala His														0243	
	Me	et As	sp Al	la Se	er Al	la Al	la Al	la I	le As	sn Va	al As	sn A	la Hi	s	
						5				:	10				
TTA	ACA	GAA	GTT	GGA	AAG	AAA	CGT	TTT	TTA	GGA	GAG	AGA	ATC	AGT	0288
Leu	Thr	Glu	Val	Gly	Lys	Lys	Arg	Phe	Leu	Gly	Glu	Arg	Ile	Ser	
	15			_	_	20					25				
CAA	AGT	TTG	AAG	GGT	AAA	GAT	CTG	AGA	GCT	CTG	TTT	TCA	AGA	ACT	0333
				Gly											
	30		-			35					40			,	
GAG	AGC	AAG	GGT	AGA	AAT	GTC	ААТ	AAA	CCT	GGG	GTT	GCA	TTT	TCT	0378
Glu	Ser	Lys	Gly	Arg	Asn	Val	Asn	Lys	Pro	Gly	Val	Ala	Phe	Ser	
	45	_				. 50					55				
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GTT	CTC	ACC	TCA	GAT	TTT	AAT	CAA	AGT	GTT	AAA	GAA	TCT	TTG	AAA	0423
Val	Leu	Thr	Ser	Asp	Phe	Asn	Gln	Ser	Val	Lys	Glu	Ser	Leu	Lys	
	60					65					70				

								_							
TAT	GAG	CCA	GCA	TTA	TTT	GAA	TCT	CCA	AAA	GCT	GAC	CCA	AAA	TAA	0468
Tyr	Glu	Pro	Ala	Leu	Phe	Glu	Ser	Pro	Lys	Ala	Asp	Pro	Lys	Asn	
	7 5					80					85			•	
											ACT				0513
Val	Ala	Ala	Ile	Val	Leu	Gly	Gly	elà	Ala	Gly	Thr	Arg	Leu	Phe	
	90					95					100				
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											CCA				0558
Pro		Thr	Ser	Arg	Arg		Lys	Pro	Ala	Val	Pro	Ile	Gly	Gly	
	105					110					115				
															0603
Сув	_	Arg	Leu	Ile	Asp		Pro	Met	Ser	Asn	Cys	He	Asn	ser	
	120					125					130				
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AAT TTT GGG GAT GGC TTT GTG GAG GTT TTT GCT GCT ACA CAA ACA 0738

175

Asn Phe Gly Asp Gly Phe Val Glu Val Phe Ala Ala Thr Gln Thr

								,							
CCT	GGA	GAA	TCA	GGA	AAG	AAA	TGG	TTC	CAG	GGC	ACC	GCT	GAT	GCA	0783
Pro	Gly	Glu	Ser	Gly	Lys	Lys	Trp	Phe	Gln	Gly	Thr	Ala	Asp	Ala	
	180					185					190			•	
GTA	AGA	CAG	TTT	TTC	TGG	GCA	TTT	GAG	GAT	TCC	AAA	TCC	AAG	GAT	0828
Val	Arg	Gln	Phe	Phe	Trp	Ala	Phe	Glu	Asp	Ser	Lys	Ser	Lys	Asp	
	195					200			•		205				
GTC	GAG	CAT	ATA	GTT	ATT	TTA	TCC	GGT	GAT	CAT	CTT	TAC	CGA	ATG	0873
Val	Glu	His	Ile	Val	Ile	Leu	Ser	Gly	Asp	His	Leu	Tyr	Arg	Met	
	210					215		-	-		220	_	_		
GAT	TAC	ATG	AGT	TTT	TGG	CAG	AAG	CAC	ATT	GAC	ACC	AAT	GCT	GAT	0918
											Thr				
F	225				•	230	-	••		-	235			_	
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ልሞሞ	ACA	GTG	TCA	TGC	АТА	ccc	ATG	GAT	GAC	AGC	CGT	GCA	TCG	GAT	0963
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	255					20U					200				
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											ATG				1053
Ala	Glu	Lys	Pro	Lys	GTA	Ser	Asp	ren	TUL	WTS	Met	GIU	val	ASP	

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ACA	ACT	GTT	CTT	GGG	CTC	TCT	GAC	CTT	GAA	GCT	ATG	TCA	AAT	CCA	1098
Thr	Thr	Val	Leu	Gly	Leu	Ser	Asp	Leu	Glu	Ala	Met	Ser	Asn	Pro	
	285					290					295			•	
TAT	ATT	GCA	TCA	ATG	GGT	GTT	TAT	GTC	TTT	CGA	ACG	GAT	GTT	CTT	1143
Tyr	Ile	Ala	Ser	Met	Gly	Val	Tyr	Val	Phe	Arg	Thr	Asp	Val	Leu	
	300					305					310				
ATG	GAG	CTT	CTC	AAT	CGA	AAA	TAC	CCT	TCA	AGC	AAT	GAT	TTT	GGC	1188
Met	Glu	Leu	Leu	Asn	Arg	Lys	Tyr	Pro	Ser	ser	Asn	Asp	Phe	Gly	
	315					320					325				
TCT	GAG	ATT	ATT	CCT	TCA	GCT	GTA	GGA	GAG	TCT	AAT	GTT	CAG	GCA	1233
Ser	Glu	Ile	Ile	Pro	Ser	Ala	Val	Gly	Glu	Ser	Asn	Val	Gln	Ala	
	330					335					340				
TAT	CTA	TTT	AAT	GAC	TAC	TGG	GAG	GAT	ATC	GGA	ACC	ATA	AAG	TCT	1278
Tyr	Leu	Phe	Asn	Asp	Tyr	Trp	Glu	Asp	Ile	Gly	Thr	Ile	Lys	Ser	
	345					350					355				
TTC	TTT	GAT	TCC	AAT	TTG	GCC	CTT	ACA	CAA	CAG	CCT	CCC	AAG	TTT	1323
Phe	Phe	Asp	Ser	Asn	Leu	Ala	Leu	Thr	Gln	Gln	Pro	Pro	Lys	Phe	
	360					365					370				
GAA	TTC	TAC	GAT	CCA	AAA	'ACA	CCT	TTT	TAT	ACA	TCT	GCA	AGA	TTT	1368

Glu Phe Tyr Asp Pro Lys Thr Pro Phe Tyr Thr Ser Ala Arg Phe

385

380

								9)						
CTG	CCT	CCT	ACA	AAA	GTC	GAC	AGG	TGC	AAG	ATT	GTC	GAT	TCC	ATT	1413
Leu	Pro	Pro	Thr	Lys	Val	Asp	Arg	Cys	Lys	Ile	Val	Asp	Ser	Ile	
	390					395					400			•	
GTA	TCC	CAT	GGT	TGT	TTT	CTA	CAG	GAG	TCT	AGC	ATC	CAA	CAT	TCC	1458
Val	Ser	His	Gly	Cys	Phe	Leu	Gln	Glu	Ser	Ser	Ile	Gln	His	Ser	
	405					410					415				
ATT	GTT	GGT	GTT	CGC	TCA	AGA	TTA	GAG	TCC	GGG	GTT	GAG	TTC	CAG	1503
Ile	Val	Gly	Val	Arg	Ser	Arg	Leu	Glu	Ser	Gly	Val	Glu	Phe	Gln	
	420					425					430				
GAC	ACC	ATG	ATG	ATG	GGC	GCA	GAT	TAC	TAT	CAA	ACT	GAA	TCA	GAA	1548
Asp	Thr	Met	Met	Met	Gly	Ala	Asp	Tyr	Tyr	Gln	Thr	Glu	Ser	Glu	
	435					440					445				
						GAG-									1593
Ile		Ser	Leu	Leu	Ala	Glu	Gly	Lys	Val	Pro	Val	Gly	Val	Gly	
	450					455					460				
						TAA		ATA			AAG		GCC	AAA	1638
Gln		Thr	Lys	Ile	Lys	Asn	Cys	Ile	Ile	Asp	Lys	Asn	Ala	Lys	
	465					470					475				
						,									
						ATC									1683
Ile	Gly	Lys	Asp	Val	Val	Ile	Ala	Asn	Thr	Asp	Gly	Val	Glu	Glu	

485

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														ACC	1728
														TAG End	1773
ATT	TAAT	CAT I	AACC!	rcat:	ra Gi	\AA G	AAAT.	A, AT	PTTG:	CATG	ATT:	TCCT	P T T		1823
CATO	GTAA	CCT 1	AAAC'	rggc	ra ai	ACCA	CGAG	G TT	rtct(CATC	TGT	ATAT	ATA		1873
ATAT	rgtc:	rat <i>l</i>	AACT!	ATGG!	LA TA	ATCT!	TAAT	A. AA	AAAA	AAAA	AAA	AAAA	AAA		1923
A															1924
		e sma						_			_	•		se ha	s
GG		ACT Thr												AGC Ser	0044
		TTC Phe													0089
ACG Thr		TTT Phe													0134
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CCG	ATT	GTT	GTC	TCT	ccc	AAA	GCT	GTT	TCT	GAT	TCT	AAG	AAT	TCG	0179
Pro	Ile	Val	Val	Ser	Pro	Lys	Ala	Val	Ser	Asp	Ser	Lys	Asn	Ser	
45		•			50					55				,	
CAG	ACT	TGT	CTT	GAC	CCT	GAA	GCC	AGC	CGT	AGT	GTT	CTT	GGT	ATT	0224
Gln	Thr	Cys	Leu	Asp	Pro	Glu	Ala	Ser	Arg	Ser	Val	Leu	Gly	Ile	
60					65					70					
ATA	CTT	GGA	GGT	ggt	GCT	GGT	ACA	CGT	CTT	TAC	CCG	TTG	ACT	AAG	0269
Ile	Leu	Gly	Gly	Gly	Ala	Gly	Thr	Arg	Leu	Tyr	Pro	Leu	Thr	Lys	•
75					80					85					
											•				
AAG	AGA	GCC	AAA	CCA	GCC	GTG	CCA	CTC	GGT	CCT	AAT	TAT	AGG	CTT	0314
Lys	Arg	Ala	Lys	Pro	Ala	Val	Pro	Leu	Gly	Ala	Asn	Tyr	Arg	Leu	
90					95					100					
ATT	GAT	ATC	CCA	GTG	AGC	AAT	TGT	TTG	AAC	AGT	AAT	TTA	TCC	AAA	0359
Ile	Asp	Ile	Pro	Val	Ser	Asn	Cys	Leu	Asn	Ser	Asn	Ile	Ser	Lys	
105					110					110					
ATA	TAT	GTT	CTT	ACA	CAA	TTC	AAT	TCT	GCT	TCT	CTG	AAT	CGT	CAT	0404
Ile	Tyr	Val	Leu	Thr	Gln	Phe	Asn	Ser	Ala	Ser	Leu	Asn	Arg	His	
115					120					125					•
					GCT										0449
Leu	Ser	Arg	Ala	Tyr	Ala	Ser	Asn	Met	Gly		Tyr	Lys	Asn	Glu	•
130					135					140					
		-													
								44 -							
					CTT -										0494
Gly	Phe	Val	Glu	Val	Leu	Ala	Ala	Gln			Pro	Glu	Asn	Pro	
145					150					155					

		12	•
AAC TGG TTT CAG			
Asn Trp Phe Gln G	Gly Thr Ala Asp	Ala Val Arg Gln	Tyr Leu Trp
160	165	170	
CTT TTC GAA GAG	CAC AAT GTT CTT	GAG TAC TTG ATT	CTT GCT GGT 0584
Leu Phe Glu Glu F	His Asn Val Leu	Glu Tyr Leu Ile	Leu Ala Gly
175	180	185	
•			
GAC CAT TTG TAT	CGA ATG GAT TAT	GAA AGA TTT GTC	CAA GCT CAC 0629
Asp His Leu Tyr A	Arg Met Asp Tyr	Glu Arg Phe Val	Gln Ala His
190	195	200	
AGA GAA ACT GAT C	GCA GAC ATT ACT	GTT GCT GCA TTG	CCA ATG GAT 0674
Arg Glu Thr Asp A	Ala Asp Ile Thr	Val Ala Ala Leu	Pro Met Asp
205	210	215	
-			
GAA AAG CGT GCT	ACT GCA TTT GGT	TTG ATG AAA ATT	GAT GAA GAA 0719
Glu Lys Arg Ala T			
220	225	230	
GGA AGA ATT ATT	GAG TTT GCC GAG	AAA CCG AAA GGA	GAA CAA TTG 0764
Gly Arg Ile Ile (
235	240	245	
233		•	
	•		
AAA GCT ATG AAG	GTT GAT ACC ACA	ATC CTG GGT CTG	GAC GAT GAG 0809
Lys Ala Met Lys			
•	255	260	<u>-</u>
250	<i>233</i>	200	

AGA	GCA	AAA	GAA	ATG	CCA	TTC	ATA	GCC	AGC	ATG	GGC	ATA	TAT	GTT	0854
-					Pro										
265	••	-1-			270					275	,		-3-		
203					2,0					2.3					
ATT	AGC	AAA	GAT	GTA	ATG	CTT	AAT	CTG	CTT	CGG	GAG	CAA	TTT	CCT	0899
Ile	Ser	Lys	Asp	Val	Met	Leu	Asn	Leu	Leu	Arg	Glu	Gln	Phe	Pro	
280					285					290					
GGT	GCT	AAT	GAT	TTT	GGA	AGT	GAA	GTT	ATT	CCA	GGC	GCC	ACT	TCC	0944
Gly	Ala	Asn	qaA	Phe	Gly	Ser	Glu	Val	Ile	Pro	Gly	Ala	Thr	Ser	
295					300					305					
					CAA										0989
Ile	Gly	Leu	Ary	Val	Gln	Ala	Tyr	Leu	Tyr		СТĀ	Tyr	Trp	GIU	
310					315					320					
CAT	ል ጥጥ	GGT	ACC	ATT	GAA	GCT	TTT	TAC	AAT	GCT	AAC	TTG	GGA	ATC	1034
					Glu										
325		,			330			-		335			-		
•															
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ACC	AAA	AAG	CCG	GTG	CCA	GAT	TTT	AGC	TTC	TAT	ĢAT	CGT	TCA	TCT	1079
Thr	Lys	Lys	Pro	Val	Pro	Asp	Phe	Ser	Phe	Tyr	Asp	Arg	Ser	Ser	
340					345					350					
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Pro	Ile	Tyr	Thr	Gln	Pro	Arg	Tyr	Leu			Ser	Lys	Met	Leu	
355					360					365					

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GAT	GCT	GAT	ATA	ACT	GAC	AGC	GTC	ATC	GGT	GAA	GGC	TGT	GTT	ATT	1169
Asp	Ala	Asp	Ile	Thr	Asp	Ser	Val	Ile	Gly	Glu	Gly	Cys	Val	Ile	
370					375					380					
							•								
										GGA				•	1214
Lys	Asn	Cys	Lys	Ile	His	His	Ser	Val	Ile	Gly	Leu	Arg	Ser	Cys	
385					390					395					
							•								
														GCT	1259
Ile	Ser	Glu	Gly	Ala		Ile	Glu	Asp	Thr	Leu	Leu	Met	Gly	Ala	
400					405					410					
														٠.	
													:	****	
										TTC					1304
Asp	Tyr	Tyr	Glu	Thr		Ala	Asp	Arg	Lys	Phe	Leu	Ala	Ala	Γλε	
415					420					425					
				3.00	001	a mm	666		201		.	500	~ m	c) m	1240
										CGT					1349
-	ser	vaı	PIO	TIE		116	СТА	ASN		Arg	TIE	GIY	wsh	wsb	
430					435					440					
													•		
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										Glu		-			1374
	пÃ2	TIE	110	non.	450	nop	non	742		455	AIG	nzu	<i></i> 9	01 4	
445					450					755					
ACA	GAC	GGA	TAC	TTC	ATA	AAG	AGC	GGA	ATA	GTC	ACT	ATA	ATC	AAG	1439
										Val					-
460	nop	1	-1-		465	-4-				470				4 -	
700						,									

GAC GCC ATG ATT CCA AGT GGA ACT GTA ATC TAG AAATGGAGCA	1482
Asp Ala Met Ile Pro Ser Gly Thr Val Ile End	
475 480 485	
TATAATAAAT ATCACTGCCT ATTTACAGTA CCTATCTGAG TCTCCCACCA	1532
TGACCCTTTG ATTCAATCTT TTAGTTATGT AAATATTTTT GGCTTTTGCG	1582
ATTTTGCCAT AAATTTGAAG AAGCGAGGAT TCAGGGACGA TAGTGCTATG	1632
AATTGGAAGA AAGGATTTGG GGGATATCTT TGTAAAGACA TTTTGACTAC	1682
TGGGCACTAA AAATTTGGTA ATGCTATACC AAAATATATA AAAAGATCTT	1732
GCTGGGTTTT GGTAAAAAA AAAAAAAAAA A	1763
· · · · · · · · · · · · · · · · · · ·	1703
The sucrose phosphate - cynthase has the following	
•	
nucleotide sequence (Seq. ID No. 3):	
GGGCTGCAGG GAAGCTCTGA ACTTCAAAA ATG GCG GGA AAT GAT	. 0044
Met Ala Gly Asn Asp	
5	
	•
TGG ATA AAC AGT TAT TTA GAG GCA ATT CTG GAT GTG GGT CCA GO	A 0089
Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu Asp Val Gly Pro G	ly
10 15	20
CTT GAT GAT GCA AAA TCA TCT TTG CTT TTG AGA GAA AGA GGC AG	G 0134
Leu Asp Asp Ala Lys Ser Ser Leu Leu Leu Arg Glu Arg Gly An	:q
·	35
•	
TTT AGT CCT ACT CGT TAC TTT GTT GAA GAA GTT ATC ACT GGT TT	TT 0179
Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu Val Ile Thr Gly Ph	je
40 45 5	50

								1	6						
					CAT										0224
Asp	Glu	Thr	Asp		His	Arg	Ser	Trp	Val	Arg	Ala	Gln	Ala	Thr	
				55					60					65	
AGG	AGT	CCT	CAA	GAG	AGG	AAT	ACT	AGA	TTG	GAG	AAC	ATG	TGT	TGG	0269
Arg	Ser	Pro	Gln	Glu	Arg	Asn	Thr	Arg	Leu	Glu	Asn	Met	Cys	Trp	
	•	•		70					. 75					80	
										•					
					GCT										0314
Arg	Ile	Trp	Asn	Leu	Ala	Arg	Gln	Lys	Lys	Gln	Leu	Glu	Asn	Glu	
				85					90					95	
GAA	GCT	CAG	CGG	AAG	ACA	AAA	CGT	CGT	ATG	GAG	CTT	GAG	AGG	GGT	0359
Glu	Ala	Gln	Arg	Lys	Thr	Lys	Arg	Arg	Met	Glu	Leu	Glu	Arg	Gly	
				100					105		٠			110	
CGT	CGA	GAA	GCA	ACT	GCT	GAT	ATG	TCG	GAG	GAC	TTA	TCA	GAA	GGC	0404
Arg	Arg	Glu	Ala	Thr	Ala	Asp	Met	Ser	Glu	Asp	Leu	Ser	Glu	Gly	
				115					120					125	
	•														
GAA	AAG	GAC	ATT	TCA	GCT	CAT	GGT	GAT	AGC	ACC	CGT	CCT	AGA	TTG	0449
Glu	Lys	Asp	Ile	Ser	Ala	His	Gly	Asp	Ser	Thr	Arg	Pro	Arg	Leu	
				130					135					140	
CCA	AGA	ATA	AAT	TCT	CTT	GAT	GCT	ATG	GAG	.ACA	TGG	ATT	AGT	CAA	0494
Pro	Arg	Ile	Asn	Ser	Leu	Asp	Ala	Met	Glu	Thr	Trp	Ile	Ser	Gln	
				145					150					155	
						•									
CAA	AAG	GAA	ааа	AAA	CTC	TAC	СТТ	GTT	TTG	ATA	AGT	CTT	CAT	GGT	0539
					Leu										
~·	_,_			160		_			165					170	

								1	7						
TTG	ATA	CGA	GGT	GAA	AAC	ATG	GAA	CTT	GGC	CGT	GAT	TCT	GAT	ACT	0584
Leu	Ile	Arg	Gly	Glu	Asn	Met	Glu	Leu	Gly	Arg	Asp	Ser	Asp	Thr	
				175					180					185	
					TAT			,							0629
Gly	Gly	Gln	Val		Tyr	Val	Val	Glu		Ala	Arg	Ala	Leu	Gly	
				190					195					200	
mac	a mc	CCA	CCT	سعت	TAT	a c a	C-πan	CAT	mm c	COTT N	3 CM	3.00	~ . .	~ ~ ~	0674
				•	Tyr										0674
DEL	Mec	FIU	GIY	205	ıyı	nr y	Val	лэр	210	Leu	1111	ALG		215	
				203					210					213	
TCA	TCT	CCT	GAC	GTG	GAT	TGG	AGT	TAT	GGG	GAG	CCT	ACT	GAG	ATG	0719
					Asp										
				220					225		٠.٠.	· ·		230	
CTG	AAT	CCA	AGG	GAT	TCC	AAT	GGT	TTT	GAT	GAT	GAT	GAT	GAT	GAA	0764
Leu	Asn	Pro	Arg	Asp	Ser	Asn	Gly	Phe	Asp	Asp	Asp	Asp	Asp	Glu	
				235					240					245	
				•		4									
					GGT										0809
Met	Gly	Glu	Ser		Gly	Ala	Tyr			Arg	Ile	Pro			
•				250					255				;	260	
		a		m» m	እመረገ	CCA	***	~» »	C3.C		mcc.	oéa	ma m	3 (m.c.)	0054
					ATC										0854
Pro	Arg	Asp	гÀг	1yr 265	Ile	WIG	nys			rea	Trp	Pro			
				203					270					275	

								_							
									CAC						0899
Pro	Glu	Phe	Val	Asp	Gly	Ala	Leu	Asn	His	Ile	Val	Gln	Met	Ser	
				280					285					290	
AAA	GTT	TTA	GGT	GAG	CAA	ATT	GGT.	AGC	GGG	GAA	ACA	GTT	TGG	CCA	0944
Lys	Val	Leu	Gly	Glu	Gln	Ile	Gly	Ser	Gly	Glu	Thr	Val	Trp	Pro	
				295					300					305	
GTT	GCC	ATT	CAT	GGA	CAT	TAT	GCT	GAT	GCT	GGT	GAT	TCT	GCT	GCT	0989
Val	Ala	Ile	His	Gly	His	Tyr	Ala	Asp	Ala	Gly	Asp	Ser	Ala	Ala	
				310					315					320	
CTT	CTT	TCT	GGT	GGC	CTA	AAT	GTT	CCA	ATG	CTT	TTA	ACG	GGG	CAT	1034
Leu	Leu	Ser	Gly	Gly	Leu	Asn	Val	Pro	Met	Leu	Leu	Thr	Gly	His	
				325					330					335	
TCT	CTT	GGC	CGA	GAC	AAG	TTA	GAG	CAG	CTC	CTC	AAA	CAG	GGT	CGA	1079
Ser	Leu	Gly	Arg	Asp	Lys	Leu	Glu	Gln	Leu	Leu	Lys	Gln	Gly	Arg	
				340					345					350	
ATG	TCT	AAA	GAT	GAC	ATA	AAC	AAT	ACA	TAC	AAA	ATA	ATG	CGT	AGG	1124
Met	Ser	Lys	Asp	Asp	Ile	Asn	Asn	Thr	Tyr	Lys	Ile	Met	Arg	Arg	
				355					360					365	
ATA	GAA	GCC	GAA	GAG	TTA	TCA	CTT	GAT	GCC	TCT	GAG	ATA	GTC	ATA	1169
									Ala						
				270					375					380	

PCT/EP94/01671 WO 94/28146

								7	9					•	
ACT	AGT	ACA	AGA	CAA	GAA	ATA	GAA	GAG	CAA	TGG	CAC	CTC	TAT	GAT	1214
Thr	Ser	Thr	Arg	Gln	Glu	Ile	Glu	Glu	Gln	Trp	His	Leu	Tyr	Asp	
				385					390					395	
GGG	TTT	GAT	CCT	GTG	CTA	GAA	CGT	AAA	CTC	CGT	GCT	AGG	ATG	AAG	1259
Gly	Phe	Asp	Pro	Val	Leu	Glu	Arg	Lys	Leu	Arg	Ala	Arg	Met	Lys	
				400					405					410	
								•							
CGT	GGT	GTA	AGC	TGT	TAT	GGA	AGG	TTC	ATG	CCC	CGG	ATG	GTT	GTT	1304
Arg	Gly	Val	Ser	Cys	Tyr	Gly	Arg	Phe	Met	Pro	Arg	Met	Val	Val	
				415					420					425	
ATT	CCT	CCT	GGA	ATG	GAA	TTC	AAT	CAT	ATT	GTT	CCA	CAT	GAG	GGT	1349
Ile	Pro	Pro	Gly	Met	Glu	Phe	Asn	His	Ile	Val	Pro	His	Glu	Gly	
				430					435					440	
														TCA	1394
Asp	Met	Asp	Gly	Glu	Thr	·Glu	Glu	Thr	Glu	Glu	His	Pro	Thr	Ser	
				445			•		450					455	
														AAA	1439
Pro	Asp	Pro	Pro	Ile	Trp	Ala	Glu	Ile	Met	Arg	Phe	Phe	Ser	Lys	
				460					465					470	
						_									
														AAG	1484
Pro	Arg	Lys	Pro	Met	Ile	Leu	Ala	Leu	Ala	Arg	Pro	Asp	Pro	Lys	

480

485

								2	0						
AAG	AAT	ATC	ACG	ACT	TTG	GTC	AAA	GCA	TTT	GGA	GAA	TGC	CGT	CCA	1529
Lys	Asn	Ile	Thr	Thr	Leu	Val	Lys	Ala	Phe	Gly	Glu	Cys	Arg	Pro	
				490					495					500	
CTA	AGG	GAG	CTA	GCT	AAT	CTT	ACT	CTT	ATA	ATG	GGT	AAC	CGA	GAT	1574
Leu	Arg	Glu	Leu	Ala	Asn	Leu	Thr	Leu	Ile	Met	Gly	Asn	Arg	Asp	
				505					510					515	
GGT	ATT	GAC	GAG	ATG	TCA	AGC	ACC	AGT	TCT	TCA	GTT	CTC	CTG	TCA	1619
Gly	Ile	Asp	Glu	Met	Ser	Ser	Thr	Ser	Ser	Ser	Val	Leu	Leu	Ser	
				520					525					530	
						CAA									1664
Val	Leu	Lys	Leu		Asp	Gln	Tyr	Asp		Tyr	Gly	Gln			
				535				•	540					545	
						•									
				0 10			~~	63 M	c mm	000	~>~	***		00m	1700
						CAA									1709
Tyr	Pro	ьуs	HIS		гуs	Gln	Ald	ASP		PFO	GIU	116	_	_	
				550					555					600	
mmc	CC3	CCN	N N C	7C7	AAC	GGA	CTC	արարար	አ ጥጥ	እልጥ	CCA	ሮርጥ	للحلحك	እጥጥ	1754
						Gly									1/24
Ten	AIG	VIG	цуз	605	Lys	ury	•	1	610	<i>x</i> 1011	110	****		615	
														-13	
GAG	CCA	TTT	GGG	CTG	ACT	CTA	ATA	GAG	GCA	GCA	GCT	CAT	GGT	TTA	1799

Glu Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala His Gly Leu

625

								2	1						
CCG	ATG	GTT	GCT	ACG	AAA	AAT	GGA	GGC	CCT	GTT	GAT	ATC	CAG	AGG	184
Pro	Met	Val	Ala	Thr	Lys	Asn	Gly	Gly	Pro	Val	Asp	Ile	Gln	Arg	
				635					640					645	
GTC	Cdrdr	САТ	ልልጥ	GGT	Cuhin	CTPTP	GTG.	ርልጥ	ССТ	_ር ል ጥ	G y C	CAC	CAG	TCT	1889
											Glu				100
			•;	650					655				••••	660	
				•											
ATT	GCT	ACT	GCT	TTG	CTG	AAG	CTT	GTT	GCT	GAT	AAG	CAA	CTA	TGG	1934
Ile	Ala	Thr	Ala	Leu	Leu	Lys	Leu	Val	Ala	Asp	Lys	Gln	Leu	Trp	
				665					670		÷			675	
ACA	AAA	TGC	CAG	CAA	AAT	GGA	CTG	AAA	AAT	ATT	CAT	CTC	TAC	TCT	1979
Thr	Lys	Cys	Gln	Gln	Asn	Gly	Leu	Lys	Asn	Ile	His	Leu	Tyr	Ser	
				680					685					690	
ጥርር	CCA	GAG	САТ	ጥርር	AAG	ACA	ጥልሮ	ርጥል	ጥርጥ	CGA	ATA	ecc.	acm:	ጥ ሮር	2024
											Ile				2024
				695	2 -		-2-		700	5				705	
										•	•			CTT	2069
Arg	Gln	Arg	Gln		Gln	Trp	Gln			Ser	Asp	Glu	_		
				710					715				,	720	
GAC	AAT -	CAA	GAG	ССТ	GAA	TCT	CCA	AGT	GAT	TCT	TTA	AGA	GAT	ATA	2114
Asp	Asn	Gln	Glu	Pro	Glu	Ser	Pro	Ser	Asp	Ser	Leu	Arg	Asp	Ile	
				725					730					735	

22 AAG GAT ATA TCT CTA AAC CTT GAA GTT CTC GTT AGA CCG GAG AAA 2159 Lys Asp Ile Ser Leu Asn Leu Glu Val Leu Val Arg Pro Glu Lys 740 745 750 AGG GTG AAG ACG TTG AAA ATC TTG GGA TTG ATG ACA AAA GCA AAT Arq Val Lys Thr Leu Lys Ile Leu Gly Leu Met Thr Lys Ala Asn 755 760 765 TCG AGA ATG CTG TTA TGT TCA TGG TCT AAT GGT GTC CAT AAG ATG Ser Arg Met Leu Leu Cys Ser Trp Ser Asn Gly Val His Lys Met 770 775 780 CTT CGG AAG GCT CGG TTC TCT GAC AAA GTA GAT CAG GCT TCT AGT Leu Arg Lys Ala Arg Phe Ser Asp Lys Val Asp Gin Aia Ser Ser 785 790 795 AAA TAT CCA GCA TTT AGG AGG AGA AAA CTT ATA TAT GTT ATT GCT Lys Tyr Pro Ala Phe Arg Arg Lys Leu Ile Tyr Val Ile Ala 800 805 810 GTA GAC GGG GAT TAT GAA GAT GGA CTT TTT GAT ATT GTT CGG AGG 2384 Val Asp Gly Asp Tyr Glu Asp Gly Leu Phe Asp Ile Val Arg Arg

815 820 825

ATA TTT GAT GCT GCT GGC AAG GAG AAG ATT GAA GGT TCC ATC GGG Ile Phe Asp Ala Ala Gly Lys Glu Lys Ile Glu Gly Ser Ile Gly 830 835 840

5-15-14)

23 TTT ATA TTG TCA ACA TCC TAT TCT ATG CCC GAA ATT CAG AAC TAT 2474 Phe Ile Leu Ser Thr Ser Tyr Ser Met Pro Glu Ile Gln Asn Tyr 845 850 855 TTG CTA TCA AAA GGC TTC AAT CTT CAT GAT TTT GAT GCA TAT ATA Leu Leu Ser Lys Gly Phe Asn Leu His Asp Phe Asp Ala Tyr Ile 860 865 TGC AAC AGT GGG AGT GAG TTG TAC TAT TCA TCT TTG AAC TCA GAG Cys Asn Ser Gly Ser Glu Leu Tyr Tyr Ser Ser Leu Asn Ser Glu 875 880 885 GAG AGT AAT ATT ATA GCA GAT TCA GAT TAC CAT TCA CAC ATA GAG Glu Ser Asn Ile Ile Ala Asp Ser Asp Tyr His Ser His Ile Glu 890 895 900 TAC AGA TGG GGT GGA GAA GGC CTT AGA AGG ACT TTG CTT CGC TGG Tyr Arg Trp Gly Gly Glu Gly Leu Arg Arg Thr Leu Leu Arg Trp 905 910 915 GCA GCT TCC ATC ACA GAA AAA AAT GGT GAA AAC GAA GAA CAG GTT Ala Ala Ser Ile Thr Glu Lys Asn Gly Glu Asn Glu Glu Gln Val 920 925 930

ATT ACT GAA GAT GAA GAA GTT TCT ACG GGT TAT TGC TTT GCG TTT 2744

Ile Thr Glu Asp Glu Glu Val Ser Thr Gly Tyr Cys Phe Ala Phe
935 940 945

AAA	ATA	AAG	AAC	CAA	AAT	AAG	GTT	CCC	CCT	ACG	AAG	GAG	CTC	CGC	2789
Lys	Ile	Lys	Asn	Gln	Asn	Lys	Val	Pro	Pro	Thr	Lys	Glu	Leu	Arg	
				950					955				!	960	

- AAG TCA ATG AGG ATT CAA GCT CTT CGT TGC CAT GTG ATT TAC TGT 2834 Lys Ser Met Arg Ile Gln Ala Leu Arg Cys His Val Ile Tyr Cys 965 970 975
- CAG AAC GGA TCT AAA ATG AAT GTG ATT CCA GTA CTA GCA TCC CGT 2879
 Gln Asn Gly Ser Lys Met Asn Val Ile Pro Val Leu Ala Ser Arg
 980 985 990
- TCT CAA GCC CTC AGG TAT CTT TAT GTT CGT TGG GGA GTT GAG TTG 2924 Ser Gln Ala Leu Arg Tyr Leu Tyr Val Arg Trp Gly Val Glu Leu 995 1000 1005
- TCG AAG ATG GTT GTC TTT GTT GGA GAA TGT GGT GAC ACA GAT TAT 2969
 Ser Lys Met Val Val Phe Val Gly Glu Cys Gly Asp Thr Asp Tyr
 1010 1015 1020
- GAA GGC TTG CTT GGC GGG GTC CAT AAA ACC GTA ATA CTG AAG GGA 3014 Glu Gly Leu Leu Gly Gly Val His Lys Thr Val Ile Leu Lys Gly 1025 1030 1035
- GTC TCC AAC ACT GCT TTA AGG TCT CTC CAT GCC AAC AGA AGT TAC 3059

 Val Ser Asn Thr Ala Leu Arg Ser Leu His Ala Asn Arg Ser Tyr

 1040 1045 1.050

		CCC AAT ATT GGC GAG : Pro Asn Ile Gly Glu	3104
1055	1060	1065	

GTG AGC AAA GGG TGC AGC AGC TCC GAG ATC CAG TCC ATC GTC ACA 3149
Val Ser Lys Gly Cys Ser Ser Ser Glu Ile Gln Ser Ile Val Thr
1070 1075 1080

AAA CTC TCC AAA GCT TAA TCAGATATCT GCTGCTTTCT TTTGGGTAAG 3197
Lys Leu Ser Lys Ala End
1085

CAAGGTTTCA	TCTTATATGA	TTATATCATA	AGATACTATA	TAAGCACCTT	3247
ATTGGTAAGT	CAGTCCCATA	ATAATAATGT	ACTTCAGAAC	CACAATACTT	3297
AAAAGTTGGT	TCAGTAGTGA	TTAGTCTCAT	AATAATCATA	TAATTACACA	3347
TCCGCTGTTA	ACTAGTGGTA	ATATCTAAGC	TCAACAATAA	AGATGTAAAA	3397
TGCTAGTATG	GAAATGAATT	GCTAGCTGTT	GATCTCTTTC	CCTTTATTCT	3447
GTATTATTTC	TTTCCTCATC	TCATGTAAAA	ACAATTTTCT	GAAGGTGTAC	3497
AGTTTTTTCC	CCTTATATAT	CTGTATTATT	TCTACTATTT	TTTGTTTGTA	3547
AGAATATCCT	CTCATCGAGG	AGTGATAATT	AAATAACCGG	CTTGCTAAAT	3597
ATAAAGCTTA	TTCGAGTTAA	АААААААА	ААААААА		3635

The sucrose-synthase has the following nucleotide sequence (Seq. ID No. 4):

CT	GCA	GGA	GGG	AAA	CAA	ATT	CTT	AGC	GAT	GGC	CCG	TTT	AGC	GAA	0044
	Ala	Gly	Gly	Lys	Gln	Ile	Leu	Ser	Asp	Gly	Pro	Phe	Ser	Glu	
					5			•		10					
											•	•			
GTT	CTT	AGG	TCT	GCT	CAG	GAA	GCA	ATA	GTT	GTT	CCT	CCC	TTT	GTT	0089
Val	Leu	Arg	ser	Ala	Gln	Glu	Ala	Ile	Val	Val	Pro	Pro	Phe	Val	
15					20					25			•		
GCT	ATA	GCA	GTC	CGT	CCA	AGA	CCT	GGA	GTT	TGG	GAA	TAT	GTT	CGT	0134
Ala	Ile	Ala	Val	Arg	Pro	Arg	Pro	Gly	Val	Trp	Glu	Tyr	Val	Arg	
30					`35					40					
									CAG						0179
Val	Acn	Val	Ser	Glu	Leu	Asn	Val	Glu	Gln	Leu	Thr	Val	Ser	Glu	
45					50					55					
						•						٠.			
									GAT						0224
Tyr	Leu	His	Phe	Lys	Glu	Glu	Leu	Val	Asp	Gly	Lys	Ala	Asp	Asp	
60					65					70					
														GTT	0269
His	Tyr	Val	Leu	Glu	Leu	Asp	Phe	Glu	Pro		Asn	Glu	Ser	Val	
75					80					85					
					•										
									GGT						0314
Pro	Arg	Pro	Thr	Arg	Ser	Ser	.Ser	Ile	Gly		Gly	Val	Gln	Phe	
90					95					100					
									TTC						0359
Leu	Asn	Arg	His	Leu		Ser	Ser	Met	Phe		Asn	Lys	Asp	cys	
105					110					115					

TTG	GAG	CCG	TTA	CTT	GAT	TTT	CTT	AGA	GTG	CAC	AAA	CAT	AAA	GGA	0404
Leu	Glu	Pro	Leu	Leu	Asp	Phe	Leu	Arg	Val	His	Lys	His	Lys	Gly	
120					125					130					
GTT	GTC	ATG	ATG	TTG	AAT	GAT	CGG	ATA	CAG	ACT	ATC	CAG	CGT	CTT	0449
Val	Val	Met	Met	.Leu	Asn	Asp	Arg	Ile	Gln	Thr	Ile	Ġlņ	Arg	Leu	
135					140					145					
										CTT					0494
	Ser	Ala	Leu	Ser		Ala	Glu	Asp	Tyr	Leu	Ile	Lys	Leu	Pro	
150					155					160					
			•												
				 -											
										GTA					0539
	ASP	TUL	Pro	TYF		GIU	Pne	GIU	Pne	val	TTE	GIN	Gly	Met	
165					170					175					
GCT	ահեռեւ	GAA	AGA	GGC	TGG	GGT	GAT	аст	ccr	GAA	AGG	ىلىلىت	СТА	CAA	0564
										Glu					0504
180			5	1	185	,	p			190	•••		Dea	014	
ATG	ATG	CAT	CTA	CTA ·	CTA	GAT	ATC	CTT	CAG	GCT	ccc	GAT	CCG	TCT	0629
										Ala					
195					200	_				205		•			
					•	•									
ACA	TTA	GAG	ACA	TTT	CTG	GGA	AGA	CTT	ccc	ATG	GTG	TTT	AAT	GTG	0674
Thr	Leu	Glu	Thr	Phe	Leu	Gly	Arg	Leu	Pro	Met	Val	Phe	Asn	Val	
210					215			•		220					

GTC	ATT	TTG	TCT	GTA	CAT	GGA	TAT	TTT	GGA	CAG	GCA	CAT	GTG	CTC	0719
Val	Ile	Leu	Ser	Val	His	Gly	Tyr	Phe	Gly	Gln	Ala	His	Val	Leu	
225					230					235					
GGC	TTG	CCT	GAC	ACT	GGT	GGG	CAG	ATA	GTT	TAT	ATA	CTT	GAC	CAA	0764
Gly	Leu	Pro	Asp	Thr	Gly	Gly	Gln	Ile	Val	Tyr	Ile	Leu	Asp	Gln	
240					245					250					
														CAA	0809
	Arg	Ser	Leu	Glu		Glu	Met	Leu	Gln	Arg	Ile	Lys	Lys	Gln	
255					260					265					
CCA	സമ	CAT	GTG	ልሮጥ	רכידי	AGA	ידייני	ىلىلى	እ ጥር	GTG	ልርጥ	ccc	ጥጥር	ATT	0854
										Val					0051
270	200	1125			275	5				280		•••			
2.0		•	÷												
CCT	GAC	GCT	AAA	GGG	ACC	ACG	TGC	AAT	CAA	CGT	ATG	GAG	AAA	GTC	0899
Pro	Asp	Ala	Lys	Gly	Thr	Thr	Cys	Asn	Gln	Arg	Met	Glu	Lys	Val	
285					290					295					
AGT	GGA	ACA	GAG _.	CAT	GCT	AGT	ATC	CTG	AGA	GTT	CCT	TTC	CGA	TCA	0944
Ser	Gly	Thr	Glu	His	Ala	Ser	Ile	Leu	Arg	Val	Pro	Phe	Arg	Ser	
300					305					310					
						-									
						•									
										AGA					0989
	Lys	Gly	Ile	Leu		Lys	Trp	Ile		Arg	Phe	Asp	Val	Trp	
315					320					325					

CCT	TAT	TTA	GAG	ACC	TTC	ACT	GAG	GAT	GCA	GCT	GGT	GAA	ATT	ATT	1034
Pro	Tyr	Leu	Glu	Thr	Phe	Thr	Glu	Asp	Ala	Ala	Gly	Glu	Ile	Ile	
330					335					340					
GGC	GAG	TTG	CAG	GGT	CGT	CCA	GAT	CTG	ATA	ATT	GGC	AAC	TAC	AGC	1079
Gly	Glu	Leu	Gln	Glå	Arg	Pro	Asp	Leu	Ile	Ile	Gly	Asn	Tyr	Ser	
345					350					355					
														GTC	1124
•	Gly	Asn	He	Val		Ser	Leu	Leu	Ser	His	Lys	Met	Gly	Val	
360					365					370					
ACC	CAG	ጥርር	ልልጥ	ልጥል	GCC	САТ	GCA	ጥጥር	CAC	444	እርር	AAG	יעעוד	CCA	1169
										Lys					1103
375	01	0 ,2			380					385		2,0			
3,5										303					•
GAT	TCT	GAT	ATT	TAC	TGG	AAA	AGA	TTT	GAG	GAC	AAA	TAT	CAC	TTC	1214
Asp	ser	Asp	Ile	Tyr	Trp	Lys	Arg	Phe	Glu	Asp	Lys	Tyr	His	Phe	
390					395					400					
TCG	TGT	CAA	TTT	TCA	GCT	GAC	TTG	ATG	GCA	ATG	AAT	CAT	GCT	GAT	1259
Ser	Cys	Gln	Phe	Ser	Ala	Asp	Leu	Met	Ala	Met	Asn	His	Ala	Asp	
405					410					415					
mm-c	አሙሶ	עוצוו ע	3.CC	እ ሮ መ	y Can	'ጥአ ଦ	<i>(</i> ,),),	CAC	እጥአ	GCT	ccs	N C C	226	አአጥ	1204
										Ala					1304
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420					465					420		•			

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ACT	GTT	GGT	CAA	TAT	GAA	AGC	CAT	AAG	GCC	TTT	ACT	TTT	CCG	GGG	1349
Thr	Val	Gly	Gln	Tyr	Glu	Ser	His	Lys	Ala	Phe	Thr	Phe	Pro	Gly	
435					440					445					•
					CAC										1394
Leu	Tyr	Arg	Val	Val	His	Gly	Ile	Asp			Asp	Pro	Lys	Phe	
450					455					460					
					000	663	C 3 C	3 m/c	CCC	እመድ	ma c	mm.c	CCA	WALKET	1439
					GGG										1433
	He	Val	Ser	Pro	Gly	ATG	мър	Met	AId	475	TÄT	File	PIO	rne	
465					470					4/5					
ጥርኔ	GAG	AAG	CAT	GTC	ACC	TGT	CTC	ACT	TCA	CTT	CAT	AGA	CTT	ATA	1484
					Thr										
480		2,2			485	- 4				490		-			
														•	
					• •										
GAG	CAG	CTC	CTA	TTC	AAA	CCT	GAG	CAG	AAC	GAA	GAA	CAC	ATT	GGT	1529
Glu	Gln	Leu	Leu	Phe	Lys	Pro	Glu	Gln	Asn	Glu	.Glu	His	Ile	Gly	
495					500					505					
							•								
					TCA										1574
Val	Leu	Asp	Asp	Thr	Ser	Lys	Pro	Ile	Ile	Phe	Ser	Met	Ala	Arg	
510					515					520					
							3.03	CCC	cm^	CELS	CNC	mc c	יחאמי	ccc	1610
					AAT										1619
	Asp	Arg	val	тÀг	Asn	TTG	Tnr	GTÀ	Leu	535	GIU	cys	TÄL	GTÅ	
52 5					530										

									31						
AAC	G AA'	r GC	G AA	A CT	C AG	G GAA	CTO	GC7	A AAC	CT(G GT	r GT	A GTO	GCT	1664
Lys	s Ası	n Ala	a Lys	s Le) Ar	Glu	Lev	ı Ala	a Asr	1 Lei	u Va	l Va	l Val	Ala	
540)				545	;				550	•				
														GCC	1709
Gly	туг	Asr) Asp	Val	Lys	Lys	Ser	Asn	Asp	Arc	g Glu	Glu	ı Ile	Ala	
555	i				560					565					
														AGA	1754
Glu	Ile	Glu	Lys	Met	His	Arg	Leu	Ile	Gln	Glu	Tyr	Asn	Leu	Arg	
570					575					580				_	
GGA	CAA	TTT	CGC	TGG	ATT	GCT	TCT	CAA	ACA	AAT	AGA	GTA	CGA	AAT	1799
						Ala									
585					590					595	_		_		
ĢGT	GAA	CTC	TAT	CGC	TAC	ATT	TGT	GAC	AAA	GGA	GGT	ATT	TTT	GCG .	.1844
						Ile									. 2011
600			_	_	605		-	•		610	2				
											-				
CAG	CCT	GCA	TTT	TAT	GAA	GCA	TTT	GGG	СТТ	ACA	GTT	ርጥጥ	GAA	GCC	1889
						Ala									1003
615					620			1		625		Val	GIU	Ala	
									•						
ATG	ACC	TGT	GGT	CTT	CCC	ACA '	TTT	GCT	ACC	TGC	CAC	GGT	CCT	CCA	1024
						Thr :									1934
630		-			635					540		27 Å	G.T.Ā	L7.(1	
									•	- T U					

GCT	GAG	ATT	ATA	GAA	GAC	GGT	GTT	TCA	GGA	TTT	CAT	ATC	GAT	CCA	1979
Ala	Glu	Ile	Ile	Glu	Asp	Gly	Val	Ser	Gly	Phe	His	Ile	Asp	Pro	
645					650					655					
														AAG	2024
-	His	Ala	Asp	Gln		-	Lys	Met	Thr			Phe	Val	Lys	
660					665					670					
mc.c) C)	GAG	CAT	CCA	220	ייאר	TCC	እ ርማ	222	እጥር	m cm	COX	CCA	GGG	2060
														GGG	2069
675	9				680	-1-		****	<i>_</i>	685	561	n.a	GIY	GIY	
TTA	CTA	AGG	ATC	AAA	GAA	AGA	TAT	ACC	TGG	CAA	AAG	TAT	тст	GAA	2114
Leu	Leu	Arg	Ile	Lys	Glu	Arg	Tyr	Thr	Trp	Gir	Lys	Tyr	Ser	Glu	
690					695					700					
					GCA										21.59
	Leu	Met	Thr	Leu	Ala	Gly	Val	Tyr	_		Trp	Lys	Tyr	Val	
705					710					715					
nem	222	ረ ሞ አ	CAC	202	202	CAC	303		ccm	m v m	~~~	63.6	1 mc	55	
					AGA Arg										2204
720		Tie ra	GIU	an g	725	GIU	1111	ary	_	730	Leu	GIU	nec	Phe	
720					. 23	•				.50					
									•						
						•									
CAC	ATT	TTG	AAG	TTC	CGT	GAT	CTG	GCC	AAC	TCT	GTT	CCG	CTG	GCA	2249
yr	Ile	Leu	Lys	Phe	Arg	Asp	Leu	Ala	Asn	Ser	Val	Pro	Leu	Ala	
225					740					745					

ACA	GAT	GAA	GAG	CCT	TCT	ACT	ACT	GAT	GCA	GTT	GCG	ACA	TTC	CGT	2294
Thr	Asp	Glu	Glu	Pro	Ser	Thr	Thr	Asp	Ala	Val	Ala	Thr	Phe	Ara	
750					755					760					
GGA Gly			ACGC	CTGCT	'GC I	TACI	'GAGG	T TC	CAAG	TTGT	GTA	TATA	ATTA.		2343
CTGT	GAAA	GG A	ATAA	GTGT	A GC	TACA	CAAA	AGG	TTCT	CAA	CTAT	TAGT	AT		2393
CTTC	TCTG'	TG T	'AAAT	AACG	A GA	GTGA	AAAA	TGT	AATA'	TTG '	TTGA	TGTC	TT		2443
GAAA	ACTG	AG T	TTGC	TTTG	T TT.	ATTT	TTAA	GTG	TATG	ACA Z	ATAT	GTAT	CA		2493
(ATA)	ACGG2	AT T	CTTC	AGTG	A TC.	ATAT	CAAA	AAC	PACTO	GAC (CATC	GAAG!	PΤ		2543
\ATG/	LAAAI	rc G	ACAG	CAAC	A							÷	•		2563

- These sequences can also be combined together in a suitable plasmid which leads to a combination of the individual characteristics, conditioned by the expression of the protein.
- The promoter should ensure that the foreign gene is expressed in the plant. The promoter can be so chosen that the expression occurs only in specified tissues, at a determined time point in the plant's development or at a time point determined by outside influences. The promoter can be homologous or heterologous to the plant. Suitable promoters are e.g. the promoter of the 35S RNA of the cauliflower mosaic virus, the patatin promoter B33 (Rocha-Sosa et al. (1989) EMBO J 8: 23-29) or a promoter

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that ensures an expression only in photosynthetically active tissues. Other promoters can be used which ensure an expression only in specified organs, such as the root, tuber, seed, stem or specified cell types such as mesophyllic, epidermal or transport cells.

The coding sequences described herein contain the information for the formation of an mRNA for the large subunit of the ADP-glucose-pyrophosphorylase and the sucrose-phosphate-synthase (SPS) and a part of the information for formation of the small subunit of the ADP-glucose-pyrophosphorylase as well as the sucrose-synthase, that are suitable for the formation of anti-sense RNA to the corresponding genes. Whether a translatable mRNA or an anti-sense nucleic acid is formed, depends on the orientation of the coding sequence in relation to the promoter. If the 3' end of the coding sequence is fused to the 3' end of the promoter, an anti-sense RNA results, and by fusion of the 5' end of the coding to the 3' end of the promoter a translatable RNA results. This latter leads to an increase of the enzyme activity in the cell, whilst the first leads to a reduction of the enzyme activity in the cell.

The coding sequence for the large and small subunit of the ADP-glucose-pyrophosphorylase, the sucrose phosphate synthase and the sucrose synthase can be one of those described in this invention or can be one that is derived by modifications of the sequences described above.

Thereby especially modifications of the sequences can be considered which lead to by-passing of the plant's own regulation mechanisms. Modifications to the DNA sequences of the invention can be by known methods, such as e.g. base exchange or targeted or non-targeted mutagenesis. The

35 so-formed derivatives of the DNA sequences of the invention are also within the scope of the invention.

With plasmids, which contain one or more of the DNA sequences of the invention, sugar beet can be transformed with the object of raising and/or reducing the enzyme activity and/or the change of the sucrose concentration.

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For the introduction of the DNA sequences of the invention in sugar beet, a large number of cloning vectors are available, which contain a replication signal for E. coli and a marker, which allows a selection of the transformed cells.

According to the introduction method of the desired gene in the plant, other DNA sequences may be suitable. Should the Ti- or Ri-plasmid be used, e.g. for the transformation of the plant cell, then at least the right boundary, often however both the right and left boundary of the Ti- and Ri-Plasmic T-DNA, is attached, as a flanking region, to the gene being introduced. The use of T-DNA for the transformation of plants cells has been intensively researched and is well described in EP 120 516; Hoekama, In: The Binary Plant Vector System, Offset-drukkerij Kanters B.V. Alblasserdam, (1985), Chapter V; Fraley, et al., Crit. Rev. Plant Sci., 4:1-46 and An et al. (1985) EMBO J. 4: 277-287. Once the introduced DNA is integrated in the genome, it is as a rule stable there and remains 25 also in the offspring of the original transformed cells. It normally contains a selection marker, which induces resistance in the transformed plant cells against a biocide or antibiotic such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricin etc. The individual marker employed should therefore allow the selection of transformed cells from cells, which lack the introduced DNA.

For the introduction of DNA into a plant, besides 35 transformation using Agrobacteria, there are many other

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techniques available. These techniques include the fusion of protoplasts, microinjection of DNA and electroporation. as well as ballistic methods and virus infection. From the transformed plant material, whole plants can be regenerated in a suitable medium, which contains antibiotics or biocides for the selection. The resulting plants can then be tested for the presence of introduced DNA. No special demands are placed on the plasmids in injection and electroporation. Simple plasmids, such as e.g. pUC-derivatives can be used. Should however whole plants be regenerated from such transformed cells the presence of a selectable marker gene is necessary. The transformed cells grow within the plants in the usual manner (see also McCormick et al. (1986) Plant Cell Reports 5: 81-84). These plants can be grown normally and crossed with plants, that possess the same transformed genes or different. The resulting hybrid individuals have the corresponding phenotypical properties.

In order to understand the examples forming the basis of this invention all the processes necessary for these tests and which are known per se will first of all be listed:

25 1. Cloning process

The vectors pUC 18/19 and M13mp10 series (Yanisch-Perron et al. (1985) Gene 33: 103-119), as well as the vector EMBL 3 (Frischauf et al. (1983) J Mol Biol 170: 827-842) were used for cloning.

For the plant transformations, the gene constructs were cloned in the binary vector BIN 19 (Bevan (1984) Nucl. Acids Res 12: 8711-8720)

Bacterial strains

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The E. coli strain BMH71-18 (Messing et al., Proc. Natl. Acad. Sci. USA (1977), 24, 6342-6346) or TB1 was used for the pUC and M13 mP vectors.

For the vector BIN19, the *E. coli* strain TB1 exclusively, was used. TB1 is a recombinant-negative, tetracycline-resistant derivative of strain JM101 (Yanisch-Perron et al., Gene (1985), 33, 103-119). The genotype of the TB1 strain is (Bart Barrel, personal communication):

10 F'(traD36, proAB, lacI, lacZΔM15), Δ(lac, pro), SupE,
thiS, recA, Sr1::Tn10(TcR).

The transformation of the plasmids into the potato plants was carried out using Agrobacterium tumefaciens strain

LBA4404 (Bevan, (1984), Nucl. Acids Res. 12, 8711-8720).

3. Transformation of Agrobacterium tumefaciens

In the case of BIN19 derivatives, the insertion of the DNA into the Agrobacterium was effected by direct transformation in accordance with the method of Holsters et al., (1978) (Mol Gene Genet 163: 181-187). The plasmid DNA of the transformed Agrobacterium was isolated in accordance with the method of Birnboim and Doly (1979)

(Nucl Acids Res 7: 1513-1523) and was analysed by gel electrophoresis after suitable restriction cleavage.

4. Sucrose phosphate-synthase activity test

30 The sucrose phosphate-synthase activity was determined according to the method of Siegel and Stitt (1990, Plant Science 66: 205-210) in a two stage analysis. To 180 μ l of a solution of 50mM HEPES/KOH (pH 7.4), 5mM magnesium chloride, 5mM fructose-6-phosphate, 25mM

glucose-6-phosphate and 6mM uridine-5'-diphosphoglucose, 20 μ l of probe was added and incubated for 10 minutes at

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25°C. It was heated for 3 minutes at 95°C, to complete the reaction. After centrifuging, the supernatant was spectroscopically analysed for the liberation of uridine-5'-diphosphate, whereby a pyruvate-kinase coupling enzyme reaction was used. Preparations without hexose phosphate, as well as the measurement of the recovery of added uridine-5'-diphosphate act as controls.

Examples

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Example 1

Cloning of cDNA to large and small subunits of the ADP glucose pyrophosphorylase of sugar beet.

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From the storage roots of 3-4 month old sugar beet plants grown in the greenhouse, RNA was isolated according to the method of Logemann et al (1987, Anal Biochem 163, 16-20). Resulting from poly-A+-RNA, a cDNA library was laid down according to the method of Gubler and Hoffmann (1983, Gene 25, 263) in the expression vector Lambda Zap II XR. To this there was used an oligo-dT primer provided with an XhoI recognising position and for synthesis of the first cDNA strand methylated cytidine nucleotide was inserted. After synthesis of the two strands an EcoRI-adaptor was attached and removed to one side by again cutting with the restriction endonuclease XhoI. In this way the hemimethylation of cDNA was hindered so that an internal XhoI recognition position is cut. By these procedures there is obtained a population of cDNA molecules, that can be cloned directed into the EcoRI/XhoI cut DNA of the phage Lambda. After packing of recombinant phage-DNA in phage heads, 200000 plaque forming units of the bank were plated out for infection of a bacterial colony and then each is probed with the total cDNA fragment of the large and/or subunit of the AGPase of potato (Müller-Roeber et

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al., 1990, MGG 224, 136-146) as an EcoRI-fragment. The recombinant phages corresponding to the hybridising signal were isolated. By in vivo excision plasmids were cut out from the Lambda zap-genome, which carry a double stranded cDNA as insertion. The plasmids were transformed in bacterial cells. The plasmid-DNA was then propagated in the bacteria. After checking the size of the insertions individual clones were analysed by determination of the primary sequence.

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Example 2

Cloning of cDNA to sucrose-phosphate-synthase (SPS) from sugar beet.

From the storage roots of 3-4 month old sugar beet plants grown in the greenhouse, RNA was isolated according to the method of Logemann et al (1987, Anal Biochem 163, 16-20). Resulting from poly-A+-RNA, a cDNA library was laid down 20 according to the method of Gubler and Hoffmann (1983, Gene 25, 263) in the expression vector Lambda Zap II XR. To this there was used an oligo-dT primer provided with an XhoI recognising position and for synthesis of the first cDNA strand methylated cytidine nucleotide was inserted. 25 After synthesis of the two strands an EcoRI-adaptor was attached and removed to one side by again cutting with the restriction endonuclease XhoI. In this way the hemimethylation of cDNA was hindered so that an internal XhoI recognition position is cut. By these procedures 30 there is obtained a population of cDNA molecules, that can be cloned directed into the EcoRI/XhoI cut DNA of the phage Lambda. After packing of recombinant phage-DNA in phage heads, 200000 plaque forming units of the bank were plated out for infection of a bacterial colony and then 35 each is probed with the total cDNA fragment of the

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sucrose-phosphate-synthase (SPS) from spinach (Sonnewald, 1992, Planta) as NotI. The recombinant phages corresponding to the hybridising signal were isolated. By in vivo excision plasmids were cut out from the Lambda zap-genome, which carry a double stranded cDNA as insertion. The plasmids were transformed in bacterial cells. The plasmid-DNA was then propagated in the bacteria. After checking the size of the insertions individual clones were analysed by determination of the primary sequence.

Example 3

Cloning of cDNA to sucrose-synthase from sugar beet.

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From the storage roots of 3-4 month old sugar beet plants grown in the greenhouse, RNA was isolated according to the method of Logemann et al (1987, Anal Biochem 163, 16-20). Resulting from poly-A+-RNA, a cDNA library was laid down according to the method of Gubler and Hoffmann (1983, Gene 25, 263) in the expression vector Lambda 2ap II XR. To this there was used an oligo-dT primer provided with an XhoI recognising position and for synthesis of the first cDNA strand methylated cytidine nucleotide was inserted. After synthesis of the two strands an EcoRI-adaptor was attached and removed to one side by again cutting with the restriction endonuclease XhoI. In this way the hemimethylation of cDNA was hindered so that an internal XhoI recognition position is cut. By these procedures there is obtained a population of cDNA molecules, that can be cloned directed into the EcoRI/XhoI cut DNA of the phage Lambda. After packing of recombinant phage-DNA in phage heads, 200000 plaque forming units of the bank were plated out for infection of a bacterial colony and then parallel is probed with both EcoRI/BgIII sub fragments sucrose synthase from maize (Worrell et al., 1991, Plant

Cell 3, 1121-1130). The recombinant phages corresponding to the hybridising signal were isolated. By in vivo excision plasmids were cut out from the Lambda zap-genome, which carry a double stranded cDNA as insertion. The plasmids were transformed in bacterial cells. The plasmid-DNA was then propagated in the bacteria. After checking the size of the insertions individual clones were analysed by determination of the primary sequence.

10 Example 4

Determination of the nucleotide sequence of the ADP glucose pyrophosphorylase, the sucrose synthase and the sucrose phosphate synthase of sugar beet and derivation of

15 the corresponding amino acid sequences

The nucleotide sequences of the insertions obtained from Examples 1 -3, were determined by standard methods by means of the dideoxy method (Sanger et al. (1977) Proc.

Natl. Acad. Sci. USA, 74, 5463-5467). The nucleotide sequences and the amino acid sequences derived therefrom are given in the sequence protocols Seq. ID No. 1-4.

The sequences are shown earlier; the protocols are as 25 follows:

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SEO ID NO: 1

SEQUENCE TYPE: Nucleotide with corresponding protein.

SEQUENCE LENGTH: 1924 base pairs

5 STRANDEDNESS: single

TOPOLOGY: linear
MOLECULE TYPE: cDNA

ORIGINAL SOURCE

10 ORGANISM: Beta vulgaris

IMMEDIATE EXPERIMENTAL SOURCE: cDNA library in Phage Lamda

zap

FEATURES:

15 from 206 to 1770 coding region

PROPERTIES: ADP-glucose-pyrophosphorylase, large subunit

20 SEQ ID NO: 2

SEQUENCE TYPE: Nucleotide with corresponding protein

SEQUENCE LENGTH: 1763 base pairs

STRANDEDNESS: single

25 TOPOLOGY: linear

MOLECULE TYPE: CDNA

ORIGINAL SOURCE

ORGANISM: Beta vulgaris

30 IMMEDIATE EXPERIMENTAL SOURCE: cDNA library in Phage Lamda

zap

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FEATURES:

from 3 to 1469 coding region

PROPERTIES: ADP-glucose-pyrophosphorylase, small subunit

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SEO ID NO: 3

SEQUENCE TYPE: Nucleotide with corresponding protein .

SEQUENCE LENGTH: 3635 base pairs

5 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: cDNA

ORIGINAL SOURCE

10 ORGANISM: Beta vulgaris

IMMEDIATE EXPERIMENTAL SOURCE: cDNA library in Phage Lamda

zap

FEATURES:

15 from 31 to 3164 coding region

PROPERTIES: Sucrose-phosphate-synthase

20 <u>SEQ ID NO: 4</u>

SEQUENCE TYPE: Nucleotide with corresponding protein

SEQUENCE LENGTH: 2563 base pairs

STRANDEDNESS: single

25 TOPOLOGY: linear

MOLECULE TYPE: cDNA

ORIGINAL SOURCE

ORGANISM: Beta vulgaris

30 IMMEDIATE EXPERIMENTAL SOURCE: cDNA library in Phage Lamda

zap

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FEATURES:

from 3 to 2300 coding region

PROPERTIES: Sucrose synthase

CLAIMS

1. DNA sequence with the coding region for die large subunit of ADP-glucose-pyrophosphorylase for the preparation of sugar beet with changed sucrose concentration, characterised in that the sequence has the following nucleotide sequence (Seq. ID No.1):

CAA?	\AGA}	LAA A	ACTT	CCAT	T T	CTACT	TCT	TGC	CACAI	TAT	AATT	PTCC	CAC		0050
CAAT	TTTT	rct 1	LAATT	ATTT	CT C	ACTT	CATI	TAT 1	ATCAC	TTT	TCAC	CAA	CAT		0100
rcto	SATAC	CTC C	GACAI	ACCC!	AC TI	TCT	STTCT	r cco	CAAG	ATTC	CAAJ	ACCT	CTG		0150
ATT	TCAT	TC (CACTI	AATA?	T T	MGC	TAT?	r TT	PTTT(CTGG	ATT	CAAA7	SAA		0200
AAG				CA AC						n Va					0243
_														AGT Ser	0288
				GGT Gly										ACT Thr	0333
				AGA Arg										TCT Ser	0378
						3 3 M	~ 1 1	N CM	c mm	***	C	m~m	STATE OF	***	0422

GTT CTC ACC TCA GAT TTT AAT CAA AGT GTT AAA GAA TCT TTG AAA 0423
Val Leu Thr Ser Asp Phe Asn Gln Ser Val Lys Glu Ser Leu Lys
60 65 70

								-	-						
TAT	GAG	CCA	GCA	TTA	TTT	GAA	TCT	CCA	AAA	GCT	GAC	CCA	AAA	AAT	0468
Tyr	Glu	Pro	Ala	Leu	Phe	Glu	Ser	Pro	Lys	Ala	Asp	Pro	Lys	Asn	
	75					80					85				
					CTG										0513
Val	Ala	Ala	Ile	Val	Leu		Gly	Ġly	Ala	Gly	Thr	Arg	Leu	Phe	
	90					95					100				
					AGA										0558
Pro	Leu	Thr	Ser	Arg	Arg		Lys	Pro	Ala	Val		He	GIÀ	GIÀ	
	105					110					115				
					•										
			cmc.	3 mm	GAT	CTC	CCT	እጥሮ	200	220	TICC.	እጥሮ	አስሮ	እርጥ	0603
					Asp										0003
Cys	120	Arg	Leu	TTE	vah	125	110	1100	DCI	21.01.1	130	110		001	
	120														
GGC	ATT	AGA	AAG	ATT	TTC	ATT	СТТ	ACC	CAG	TTC	AAT	TCG	TTT	TCG	0648
					Phe										
1	135	,				140					145				
CTT	AAT	CGT	CAT	CTT	GCT	CGA	ACC	TAT	AAT	TTT	GGA	GAT	GGT	GTG	0693
Leu	Asn	Arg	His	Leu	Ala	Arg	Thr	Tyr	Asn	Phe	Gly	Asp	Gly	Val	
	150					155					160				
							-								
														ACA	0738
Asn	Phe	Gly	Asp	Gly	Phe	Val	Glu	Val	Phe	Ala	Ala	Thr	Gln	Thr	

46

CCT	GGA	GAA	TCA	GGA	AAG	AAA	TGG	TTC	CAG	GGC	ACC	GCT	GAT	GCA	0783
Pro	Gly	Glu	Ser	Gly	Lys	Lys	Trp	Phe	Gln	Gly	Thr	Ala	Asp	Aļa	
	180					185					190				

GTA AGA CAG TTT TTC TGG GCA TTT GAG GAT TCC AAA TCC AAG GAT 0828 Val Arg Gln Phe Phe Trp Ala Phe Glu Asp Ser Lys Ser Lys Asp 195 200 205

GTC GAG CAT ATA GTT ATT TTA TCC GGT GAT CAT CTT TAC CGA ATG 0873

Val Glu His Ile Val Ile Leu Ser Gly Asp His Leu Tyr Arg Met .

210 220

GAT TAC ATG AGT TTT TGG CAG AAG CAC ATT GAC ACC AAT GCT GAT 0918
Asp Tyr Met Ser Phe Trp Gln Lys His Ile Asp Thr Asn Ala Asp
225
230
235

ATT ACA GTG TCA TGC ATA CCC ATG GAT GAC AGC CGT GCA TCG GAT 0963

Ile Thr Val Ser Cys Ile Pro Met Asp Asp Ser Arg Ala Ser Asp

240 245 250

TAT GGG CTG ATG AAG ATT GAT CAC ACT GGA CGC ATT GTC CAT TTT 1008

Tyr Gly Leu Met Lys Ile Asp His Thr Gly Arg Ile Val His Phe

255 260 265

GCA GAA AAA CCC AAG GGT TCT GAT CTA ACA GCA ATG CAA GTA GAT 1053
Ala Glu Lys Pro Lys Gly Ser Asp Leu Thr Ala Met Gln Val Asp
270 280

ACA	ACT	GTT	CTT	GGG	CTC	TCT	GAC	CTT	GAA	GCT	ATG	TCA	AAT	CCA	1098
Thr	Thr	Val	Leu	Gly	Leu	Ser	qzA	Leu	Glu	Ala	Met	Ser	Asn	Pro	
	285					290					295				
TAT	ATT	GCA	TCA	ATG	GGT	GTT	TAT	ĠTC	TTT	CGA	ACG	GAT	GTT	CTT	1143
Tyr	Ile	Ala	Ser	Met	Gly	Val	Tyr	Val	Phe	Arg	Thr	Asp	Val	Leu	•
	300					305					310				
ATG	GAG	CTT	CTC	AAT	CGA	AAA	TAC	CCT	TCA	AGC	AAT	GAT	TTT	GGC	1188
Met	Glu	Leu	Leu	Asn	Arg	Lys	Tyr	Pro	Ser	Ser	Asn	Asp	Phe	Gly	
	315					320					325				
												7.6			
TCT	GAG	ATT	ATT	CCT	TCA	GCT	GTA	GGA	GAG	TCT	AAT	GTT	CAG	GCA	1233
Ser	Glu	Ile	Ile	Pro	Ser	Ala	Val	Gly	Glu	Ser	Asn	Val	Gln	Ala	
	330					335					340				
TAT	CTA	TTT	AAT	GAC	TAC	TGG	GAG.	GAT	ATC	GGA	ACC	ATA	AAG	TCT	1278
Tyr	Leu	Phe	Asn	Asp	Tyr	Trp	Glu	Asp	Ile	Gly	Thr	Ile	Lys	Ser	
	345					350					355				
TTC	TTT	GAT	TCC	AAT	TTG	GCC	CTT	ACA	CAA	CAG	CCT	CCC	AAG	TTT	1323
Phe	Phe	Asp	Ser	Asn	Leu	Ala	Leu	Thr	Gln	Gln	Pro	Pro	Lys	Phe	
	360					365					370				
											TCT				1368
Glu	Phe	Tyr	Asp	Pro	Lys	Thr	Pro.	Phe	Tyr	Thr	Ser	Ala	Arg	Phe	
	375		•	•		380	٠				385				

								•	48						
CT	G CC	T CC	T AC	A AA	GTC	GAC	AGO	TG	C AA	G AT	T GT	C GA	T TC	C ATT	1413
Let	ı Pr	o Pr	o Thi	r Lys	. Val	Asp	Arç	Cys	Ly:	s Il	e Va	l As	o Se	r Iļe	
	39					395					400		•	•	
														r rcc	
Val			s Gl	Cys	Phe		Gln	. Glu	Sez	Sei	Ile	e Glr	1 His	s Ser	
	405	5				410					415				
א יחיות	CONT		1 -		mos.	161	mm x								•
														CAG	1503
116	420		AGI	Arg	ser	425	Leu	GIU	Ser	Gly			Phe	Gln	
	720					423					430				
GAC	ACC	ATG	ATG	ATG	GGC	GCA	GAT	TAC	ТАТ	CAA	АСТ	GAA	ጥርል	GAA	1548
Asp	Thr	Met	Met	Met	Gly	Ala	Asp	Tyr	Tyr	Gln	Thr	Glu	Ser	Gjn	1346
	435					440	_	_	•		445				
		•													
ATT	GCT	TCT	CTG	CTT	GCT	GAG	GGA.	AAG	GTT	CCT	GTT	GGT	GTC	GGA	1593
Ile	Ala	Ser	Leu	Leu	Ala	Glu	Gly	Lys	Val	Pro	Val	Gly	Val	Gly	
	450				•	455				•	460				
					AAG										1638
GIN		TAL	гля	TIE	Lys /		Cys	Ile	Ile			Asn	Ala	Lys	
	465					170					47C				

ATT GGA AAA GAT GTG GTA ATC GCA AAC ACG GAT GGT GTT GAG GAA 1683

Ile Gly Lys Asp Val Val Ile Ala Asn Thr Asp Gly Val Glu Glu
480

480

480

								4	9							
						GGC Gly 500				Arg					1728	
						ACC Thr 515				Gly					1773	
						AAGA									1823	
						CCAC									1873 1923	
A															1924	
2.	ADI Wii	P-gli th cl	ucos hange	e-py: ed si	roph ucro:	ospha se ca	alas once	e for	r the	pro	epara arac	ation teri	n of sed :	suga in th	nit of r beet at the No.2)	
						ACC '								•	0044	

Thr Ser Ser Lys Asn Leu Gln Asn Ser

10

CTC GCA TTC TCC TCT TCT TCT CTC TCC GGC GAC AAA ATT CAA ACG 0089 Leu Ala Phe Ser Ser Ser Leu Ser Gly Asp Lys Ile Gln Thr 15 20 25

5

ACG TCA TTT CTC AAC CGC CGA TAT TGT AGA ATC TCT TCT AGA GCT 0134 Thr Ser Phe Leu Asn Arg Arg Tyr Cys Arg Ile Ser Ser Arg Ala 30 35 40

CCG	ATT	GTT	GTC	TCT	ccc	AAA	GCT	GTT	TCT	GAT	TCT	AAG	AAT	TCG	0179
Pro	Ile	Val	Val	Ser	Pro	Lys	Ala	Val	Ser	Asp	Ser	Lys	Asn	Ser	
45					50					55					
CAG	ACT	TGT	CTT	GAC	CCT	GAA	GCC	AGC	CGT	AGT	GTT	CTT	GGT	ATT	0224
Gln	Thr	Cys	Leu	Asp	Pro	Glu	Ala	Ser	Arg	Ser	Val	Leu	Gly	Ile	
60					65					70					
					com	acm									
														AAG	0269
	Leu	GIY	GIÀ	GIA	Ala	GIÀ	Thr	Arg	Leu	_	Pro	Leu	Thr	Lys	
75					80					85					
AAG	AGA	GCC	AAA	CCA	GCC	GTG	CCA	СТС	GGT	GCT	AAT	ТАТ	AGG	СТТ	0314
					Ala										
90	-		-		95				-	100		•	,		
ATT	GAT	ATC	CCA	GTG	AGC	AAT	TGT	TTG	AAC	AGT	AAT	ATT	TCC	AAA	0359
Ile	Asp	Ile	Pro	Val	Ser	Asn	Cys	Leu	Asn	Ser	Asn	Ile	Ser	Lys	
105					110					110					
					CAA										0404
	TYT	val	Leu	Thr	Gln	hue	ASN	ser			Leu	Asn	Arg	His	
115					120					125					
CTT	TCG	CGG	GCA	TAT	GCT	AGC	AAC	ATG	GGA	GGA	TAC	AAA	ААТ	GAG	0449
														Glu	
130		· 5		- 4	135			-		140	-1-	<u>,</u> -			
•					•										
														•	
GGG	TTT	GTA	GAA	GTT	CTT	GCT	GCT	CAG	CAA	AGT	CCA	GAG	ፐፋፋ	CCA	0494
Sly	Phe	Val	Glu	Val	Leu	Ala	Ala	Gln	Gln	Ser	Pro	Glu	Asn	Pro	
145					150					155					

AAC	TGG	TTT	CAG	GGT	ACA	GCT	GAT	GCT	GTT	AGG	CAA	TAT	CTG	TGG	0539
Asn	Trp	Phe	Gln	Gly	Thr	Ala	Asp	Ala	Val	Arg	Gln	Tyr	Leu	Trp	
160					165					170					
CTT	TTC	GAA	GAG	CAC	AAT	GTT	CTT	GAG	TAC	TTG	ATT	CTT	GCT	GGT	0584
Leu	Phe	Glu	Glu	His	Asn	Val	Leu	Glu	Tyr	Leu	Ile	Leu	Ala	Gly	
175					180	-				185					
GAC	CAT	TTG	TAT	CGA	ATG	GAT	TAT	GAA	AGA	TTT	GTC	CAA	GCT	CAC	0629
Asp	His	Leu	Tyr	Arg	Met	Asp	Tyr	Glu	Arg	Phe	Val	Gln	Ala	His	
190					195				_	200					
AGA	GAA	ACT	GAT	GCA	GAC	ATT	ACT	GTT	GCT	GCA	TTG	CCA	ATG	GAT	0674
Arg	Glu	Thr	Asp	Ala	Asp	Ile	Thr	Val	Ala	Ala	Leu	Pro	Met	Asp	
205					210					215				-	
GAA	AAG	CGT	GCT	ACT	GCA	TTT	GGT	TTG	ATG	AAA	ATT	GAT	GAA	GAA	0719
Glu	Lys	Arg	Ala	Thr	Ala	Phe	Gly	Leu	Met	Lys	Ile	Asp	Glu	Glu	
220	_				225					230		-			
GGA	AGA	ATT	ATT	GAG	TTT	GCC	GAG	AAA	CCG	AAA	GGA	GAA	CAA	TTG	0764
														Leu .	
235					240			•		245					

AAA GCT ATG AAG GTT GAT ACC ACA ATC CTG GGT CTG GAC GAT GAG 0809

Lys Ala Met Lys Val Asp Thr Thr Ile Leu Gly Leu Asp Asp Gln

250 255 260

AGA	GCA	AAA	GAA	ATG	CCA	TTC	ATA	GCC	AGC	ATG	GGC	ATA	TAT	GTT	085
Arg	Ala	Lys	Glu	Met	Pro	Phe	Ile	Ala	Ser	Met	Gly	Ile	Tyr	Val	
265					270					275					
ATT	AGC	AAA	GAT	GTA	ATG	CTT	AAT	CTG	CTT	CGG	GAG	CAA	TTT	CCT	0899
Ile	Ser	Lys	Asp	Val	Met	Leu	Asn	Leu	Leu	Arg	Glu	Gln	Phe	Pro	
280					285					290					
														TCC	0944
-	Ala	Asn	Asp	Phe	Gly	Ser	Glu	Val	Ile	Pro	Gly	Ala	Thr	Ser	
295					300					305					
															
														GAG	0989
	GIY	Leu	Arg	val		УТа	Tyr	Leu	ıyr	-	GIY	Tyr	Trp	Glu	
310					315					320					
CAT	שייה ע	CCT	» CC	<u>አ</u> ጥጥ	CAA	сст	ىلىنلىك	ጥልሮ	ייי ממ	CCT	77 C	ሙጥር	GC)	ATC	1054
						Ala									1.004
325	116	GI	****		330	•••		- 7 -	A311	335	YOU	. Deu	GIY	116	•
J 2J															
ACC	AAA	AAG	CCG	GTG	CCA	GAT	TTT	AGC	TTC	TAT	GAT	CGT	TCA	TCT	1079
														Ser	
340	-	-			345	_				350	•	•			
CCA	ATT	TAT	ACA	CAA	CCT	CGG	TAT	TTG	ССТ	CCT	TCA	AAG	ATG	CTT	1124
Pro	Ile	Tyr	Thr	Gln	Pro	Arg	Tyr	Leu	Pro	Pro	Ser	Lys	Met	Leu	
355					360					365					

GAT	GCT	GAT	ATA	ACT	GAC	AGC	GTC	ATC	GGT	GAA	GGC	TGT	GTT	ATT	1169
Asp	Ala	Asp	Ile	Thr	Asp	Ser	Val	Ile	Gly	Glu	Gly	Cys	Val	Ile	
370					375					380					
AAG	AAC	TGT	AAG	ATT	CAT	CAT	TCT	GTT	ATC	GGA	CTT	CGA	TCT	TGT	1214
Lys	Asn	Cys	Lys	Ile	His	His	Ser	Val	Ile	Gly	Leu	Ar.g	Ser	Cys	
385					390				•	395					
ATC	TCG	GAG	GGT	GCA	ATC	ATT	GAG	GAC	ACA	CTG	TTG	ATG	GGA	GCT	1259
Ile	Ser	Glu	Gly	Ala	Ile	Ile	Glu	Asp	Thr	Leu	Leu	Met	Gly	Ala	
400					405					410					
GAT	TAT	TAT	GAG	ACT	GAT	GCT	GAT	CGG	AAA	TTC	CTG	GCT	GCT	AAG	1304
Asp	Tyr	Tyr	Glu	Thr	Asp	Ala	Asp	Arg	Lys	Phe	Leu	Ala	Ala	Lys	
415					420					425					
GGT	AGT	GTA	CCT	ATT	GGA	ATT	GGG	AAT	GCA	CGT	ATT	GGG	GAT	GAT	1349
Gly	Ser	Val	Pro	Ile	Gly	Ile	Gly	Asn	Ala	Arg	Ile	Gly	Asp	Asp	
430					435					440					
GTC	AAG	ATT	ATC	AAC	AGT	GAC	AAT	GTA	CAA	GAA	GCA	GCA	AGA	GAA	1394
Val	Lys	Ile	Ile	Asn	Ser	Asp	Asn	Val.	Gln	Glu	Ala	Ala	Arg	Glu	
445					450					455					
ACA	GAC	GGA	TAC	TTC	ATA	AAG	AGC	GGA	ATA	GTC	ACT	ATA	ATC	AAG	1439
Thr	Asp	Gly	Tyr	Phe	Ile	Lys	Ser	Gly	Ile	Val	Thr	Ile	Ile	Lys	
460					465					470					

GAC GCC ATG ATT CCA AGT GGA ACT GTA ATC TAG AAATGGAGCA	1482
Asp Ala Met Ile Pro Ser Gly Thr Val Ile End	
475 480 485	
TATAATAAAT ATCACTGCCT ATTTACAGTA CCTATCTGAG TCTCCCACCA	1532
TGACCCTTTG ATTCAATCTT TTAGTTATGT AAATATTTTT GGCTTTTGCG	1582
ATTTTGCCAT AAATTTGAAG AAGCGAGGAT TCAGGGACGA TAGTGCTATG	1632
AATTGGAAGA AAGGATTTGG GGGATATCTT TGTAAAGACA TTTTGACTAC	1682
TGGGCACTAA AAATTTGGTA ATGCTATACC AAAATATATA AAAAGATCTT	4720
TOGGCACIAA AAAIIIGGIA AIGCIAIACC AAAAIAIAIA AAAAGAICII	1732
GCTGGGTTTT GGTAAAAAA AAAAAAAAA A	1763
00.0001111 001.000100100100100100100100100100101010010	1703
3. DNA sequence with the coding region for sucrose	
phosphate - synthase for the preparation of sugar	-
beet with changed sucrose concentration,	
characterised in that the sequence has the follow	ing
5 nucleotide sequence (Seq. ID No.3):	_
GGGCTGCAGG GAAGCTCTGA ACTTCAAAA ATG GCG GGA AAT GAT	0044
Met Ala Gly Asn Asp	
5	
TGG ATA AAC AGT TAT TTA GAG GCA ATT CTG GAT GTG GGT CCA GGA	0089
Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu Asp Val Gly Pro Gly	
10 15 20	
CTT GAT GAT GCA AAA TCA TCT TTG CTT TTG AGA GAA AGA GGC AGG	0134
Leu Asp Asp Ala Lys Ser Ser Leu Leu Leu Arg Glu Arg Gly Arg	
25 30 35	

0179	TTT	GGT	ACT	ATC	GTT	GAA	' GAA	GTT	TTT	TAC	CGI	ACI	CCT	AGT	TTT
	Phe	Gly	Thr	Ile	Val	Glu	Glu	Val	Phe	Tyr	Arg	Thr	Pro	Ser	Phe
	50					45					40				
0224	ACA														
		Ala	Gln	Ala	Arg		Trp	ser	Arg	HIS		Asp	unr	GIU	Asp
	65					60					55	·			
0269	TGG	TGT	ATG	AAC	GAG	TTG	AGA	ACT	AAT	AGG	GAG	CÁA	CCT	AGT	AGG
••••				Asn											
	80	4				75	_				70				_
0314	GAA	AAT	GAG	CTT	CAG	AAG	AAG	CAG	CGT	GCT	TTG	AAT	TGG	ATT	AGA
	Glu	Asn	Glu	Leu	Gln	Lys	Lys	Gln	Arg	Ala	Leu	Asn	Trp	Ile	Arg
	95					90					85				
													63. 0		
0359				CTT											
	_	-	GIU	Leu	GIU		Arg	Arg	rås	THE	100	Ary	GIn	AIZ	GIU
	110	-				105					100				
0404	GGC	GAA	TCA	TTA	GAC	GAG	TCG	ATG	GAT	GCT	ACT	GCA	GAA	CGA	CGT
	Gly	Glu	Ser	Leu	Asp	Glu	Ser	Met	Asp	Ala	Thr	Ala	Glu	Arg	Arg
	125	1				120					115				
٠															
0449				CGT						•					
		-	Pro	Arg	Thr			GIÀ	HIS	АТА		TTE	Asp	Lys	GIU
	140	3				135					130				
0494	CAA	AGT	АТТ	TGG .	ACA	GAG	ATG	GCT	GAT	CTT	TCT	AAT	ATA	AGA	CCA
				Trp											
	L 5 5	1		-		150					145			-	

								5	5						
CAA	AAG	GAA	AAA	AAA	CTC	TAC	CTT	GTT	TTG	ATA	AGT	CTT	CAT	GGT	0539
Gln	Lys	Glu	Lys	Lys	Leu	Tyr	Leu	Val	Leu	Ile	Ser	Leu	His	Gly	
	-			160					165					17Ô	
										•					
TTG	ATA	CGA	GGT	GAA	AAC	ATG	GAA	CTT	GGC	CGT	GAT	TCT	GAT	ACT	0584
Leu	Ile	Arg	Gly	Glu	Asn	Met	Glu	Leu	Gly	Arg	Asp	Ser	Asp	Thr	
		_		175					180					185	
GGT	GGT	CAG	GTT	AAG	TAT	GTG	GTT	GAG	CTT	GCA	AGG	GCT	CTA	GGT	0629
Gly	Gly	Gln	Val	Lys	Tyr	Val	Val	Glu	Leu	Ala	Arg	Ala	Leu	Gly	
•	-			190					195					200	
TCG	ATG	ÇCA	GGT	GTT	TAT	AGA	GTT	GAT	TTG	CTA	ACT	AGG	CAA	GTT	0674
Ser	Met	Pro	Gly	Val	Tyr	Arg	Val	Asp	Leu	Leu	Thr	Arg	Gln	Val	
				205					210					215	
TCA	TCT	CCT	GAC	GTG	GAT	TGG	AGT	TAT	GGG	GAG	CCT	ACT	GAG	ATG	0719
Ser	Ser	Pro	Asp	Val	Asp	Trp	Ser	Tyr	Gly	Ġlu	Pro	Thr	Glu	Met	
				220					225					230	
					•										
CTG	AAT	CCA	AGG	GAT	TCC	AAT	GGT	TTT	GAT	GAT	GAT	GAT	GAT	GAA	0764
Leu	Asn	Pro	Arg	Asp	Ser	Asn	Glÿ	Phe	Asp	Asp	Asp	Asp	Asp	Glu	
				235					240					245	

ATG GGA GAG AGT AGT GGT GCT TAC ATT GTT CGT ATA CCA TTT GGG 0809 Met Gly Glu Ser Ser Gly Ala Tyr Ile Val Arg Ile Pro Phe Gly 255 260 250

PCT/EP94/01671 WO 94/28146

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								_	•						
CCG	AGG	GAT	AAG	TAT	ATC	GCA	AAA	GAA	GAG	CTT	TGG	CCC	TAT	ATT	0854
Pro	Arg	Asp	Lys	Tyr	Ile	Ala	Lys	Glu	Glu	Leu	Trp	Pro	Tyr	Ile	
				265					270					275	
		TTT													0899
Pro	Glu	Phe	Val	-	Gly	Ala	Leu	Asn	His	Ile	Val	Gln	Met	Ser	
				280					285					290	
		TTA													0944
Lys	Val	Leu	Gly		Gln	Ile	GIÀ	Ser	-	Glu	Thr	Val	•		
				295					300					305	
CIDIT	ccc	ATT	ር አጥ	CC A	ሮልሞ	ጥፈጥ	CCT	ርስጥ	CCT	CCT	C እ m	TTCTT	~~·	COM	0989
		Ile													0303
V41	nia	110		310		-3-		p	315	O±,	nap	Der		320	
				310					313					320	
CTT	CTT	TCT	GGT	GGC	CTA	AAT	GTT	CCA	ATG	CTT	TTA	ACG	GGG	CAT	1034
Leu	Leu	Ser	Gly	Gly	Leu	Asn	Val	Pro	Met	Leu	Leu	Thr	Gly	His	
				325					330					335	
•															
TCT	CTT	GGC	CGA	GAC	AAG	TTA	GAG	CAG	CTC	CTC	AAA	CAG	GGT	CGA	1079
Ser	Ļeu	Gly	Arg	Asp	Lys	Leu	Glu	Gln	Leu	Leu	Lys	Gln	Gly	Arg	
				340					345				:	350	
ATG	TCT	AAA	GAT	GAC	ATA	AAC	AAT	ACA	TAC	AAA	ATA	ATG	CGT	AGG	1124
Met	Ser	Lys	Asp	Asp	Ile	Asn	Asn	Thr	Tyr	Lys	Ile	Met	Arg	Arg	
				355					360				:	365	

								J	0						
					TTA										1169
Ile	Glu	Ala	Glu	Glu	Leu	Ser	Leu	Asp	Ala	Ser	Glu	Ile	Val	Iļe	
				370					375					380	
> com	3.CM	202	n C n	CNA	GAA	ልጥል	GAA	.eye	CAA	TGG	CAC	CTC	тат	GAT	1214
					Glu										
1111	Ser	1112	AL 9	385					390		•••			395	
				363					330					333	
															•
GGG	TTT	GAT	ССТ	GTG	CTA	GAA	CGT	AAA	CTC	CGT	GCT	AGG	ATG	AAG	1259
Gly	Phe	Asp	Pro	Val	Leu	Glu	Arg	Lys	Leu	Arg	Ala	Arg	Met	Lys	
-		-		400					405					410	
CGT	GGT	GTA	AGC	TGT	TAT	GGA	AGG	TTC	ATG	ccc	CGG	ATG	GTT	GTT	1304
Arg	Gly	Val	Ser	Cys	Tyr	Gly	Arg	Phe	Met	Pro	Arg	Met	Val	Val	
				415					420					425	
TTA	ССТ	CCT	GGA	ATG	GAA	TTC	AAT	CAT	ATT	GTT	CCA	CAT	GAG	GGT	1349
Ile	Pro	Pro	Gly	Met	Glu	Phe	Asn	His	Ile	Val	Pro	His	Glu	Gly	
				430					435			•		440	
									•						
GAT	ATG	GAT	GGT	GAA	ACA	GAA	GAA	ACT	GAA	GAG	CAT	CCT	ACA	TCA	1394
Asp	Met	Asp	Gly	Glu	Thr	Glu	Glu	Thr	Glu	Glu	His	Pro	Thr	Ser	
				445					450					455	
									-						
CCT	GAT	CCA	CCT	ATC	TGG	GCT	GAG	ATT	ATG	CGC	TTC	TTT	TCT	AAA	1439
Pro	qzA	Pro	Pro	Ile	Trp	Ala	Glu	Ile	Met	Arg	Phe	Phe	Ser	Lys	
	_			460					465					470	

								5	9						
CCA	AGG	AAG	CCA	ATG	ATA	CTT	GCC	CTT	GCT	AGG	CCT	GAC	CCG	AAG	1484
Pro	Arg	Lys	Pro	Met	Ile	Leu	Ala	Leu	Ala	Arg	Pro	Asp	Pro	Lys	
				475					480					485	
AAG	AAT	ATC	ACG	ACT	TTG	GTC	AAA	GCA	TTT	GGA	GAA	TGC	CGT	CCA	1529
Lys	Asn	Ile	Thr	Thr	Leu	Val	Lys	Ala	Phe	Gly	Glu	Cys	Arg	Pro	
				490					495					500	
					AAT										1574
Leu	Arg	Glu	Leu		Asn	Leu	Tnr	Leu		Met	GIÀ	ASN			
				505					510					515	
	•														
CCT	ን ጥጥ	CAC	GAG	ልጥር	TCA) AGC	ACC	A Crit	ጥርጥ	ጥሮኔ	ርጥጥ	כדיר	CTIC	ጥሮል	1619
					Ser										1017
GIY	116	vəh	GIG	520	Jer	DC1	1111		525	JC2		204		530	
				J											
GTG	CTT	AAG	CTA	ATT	GAT	CAA	TAC	GAC	CTT	TAT	GGT	CAA	GTA	GCA	1664
					Asp										
		_		535					540					545	
TAC	ccc	AAA	CAT	CAC	AAG	CAA	GCT	GAT	GTT	CCT	GAG	ATT	TAT	CGT	1709
Tyr	Pro	Lys	His	His	Lys	Gln	Ala	Asp	Val	Pro	Glu	Ile	Tyr	Arg	
				550					555		•			600	
TTG	GCA	GCA	AAG	ACA	AAG	GGA	GTC	TTT	ATT	AAT	CCA	GCT	TTT	AŢT	1754
T 011	717	λla	Tur	ጥኮሎ	1.00	Clv	Val	Phe	Tle	Asn	Pro	Ala	Phe	Tle	

615

				6	0				
			CTA Leu					TTA Leu 630	1799
			AAT Asn				Gln		1844
			CTT Leu				Gln		1889
			AAG Lys				Leu		1934
			GGA Gly				Tyr		1979
			ACA Thr	Leu			Ser		2024
		•	•						

AGA CAA AGG CAA CCA CAG TGG CAA AGA AGT AGT GAT GAA GGG CTT 2069

715

720

Arg Gln Arg Gln Pro Gln Trp Gln Arg Ser Ser Asp Glu Gly Leu

								6.	ı						
			GAG												2114
Asp	Asn	Gln	Glu	Pro	Glu	Ser	Pro	Ser	Asp	Ser	Leu	Arg	Asp	Ile	
				725					730				•	735	
													22.5		
			TCT										-		2159
Lys	Asp	Ile	Ser	Leu	Asn	Leu	Glu			Val	Arg	Pro			
				740					745					750	
				mmc.	122	N.T.C	mm/c	CCN	ماسان	እጥር	እሮክ	**	CC)	አ አ ጥ	2204
			ACG												2204
Arg	Val	Lys	Thr		Lys	TTE	Leu			Met	THE	rys			
				755					760					765	
mac	3.63	አ መር	CTG	מיזיים	ጥርጥ	ጥሮል	TGG	ጥርጥ	ልልጥ	CCT	GTC	САТ	AAG	ATG	2249
			Leu												
ser	Arg	Mec	Leu		cys	DEI	11p		775	O ₁	***	•••		780	
				770					773					,00	
CTT	CGG	AAG	GCT	CGG	TTC	TCT	GAC	AAA	GTA	GAT	CAG	GCT	TCT	AGT	2294
			Ala												
	5			785					790					795	
AAA	TAT	CCA	GCA	TTT	AGG	AGG	AGA	AAA	CTT	ATA	TAT	GTT	ATT	GCT	2339
Lys	Tyr	Pro	Ala	Phe	Arg	Arg	Arg	Lys	Leu	Ile	Tyr	Val	Ile	Ala	
-	_			800					805					810	
									•						
			GAT												2384
Val	Asp	Gly	Asp	Tyr	Glu	Asp	Gly	Leu	Phe	Asp	Ile	Val	Arg	Arg	
				815					820					825	

								٠.	•						
ATA	TTT	GAT	GCT	GCT	GGC	AAG	GAG	AAG	ATT	GAA	GGT	TCC	ATC	GGG	2429
Ile	Phe	Asp	Ala	Ala	Gly	Lys	Glu	Lys	Ile	Glu	Gly	Ser	Ile	GjA	
				830					835				;	B40	
Jalalı	ATA	TTG	TCA	ACA	TCC	TAT	TCT	ATG	CCC	GAA	ATT	CAG	AAC	TAT	2474
Phe	Ile	Leu	Ser	Thr	Ser	Tyr	Ser	Met	Pro	Glu	Ile	Gln	naA	Tyr	
2				845					850					855	
ጥጥር	СТА	тса	AAA	GGC	TTC	AAT	CTT	CAT	GAT	TTT	GAT	GCA	TAT	ATA	2519
Len	T.e.11	Ser	Lvs	Glv	Phe	Asn	Leu	His	Asp	Phe	Asp	Ala	Tyr	Ile	
Den		•••	-1-	860					865					870	
mcc.	220	እርጥ	ccc	AGT	GAG	TTG	TAC	TAT	TCA	TCT	TTG	AAC	TCA	GAG	2564
													Ser		
Cys	ASII	Ser	GIJ	875			-1-	-3	880					885	
				075											
CNC	3.CM	አአጥ	א מייני	ልሞል	GCA	GAT	TCA	GAT	TAC	CAT	TCA	CAC	ATA	GAG	2609
GAG	AGI	yen	Tle	Tle	Ala	Asp	Ser	Asp	Tyr	His	Ser	His	Ile	Glu	
Glu	Ser	ASII	116	890					895					900	
				030					•						
					~3 X	ccc	ر م	aca	AGG	АСТ	ጥጥር	CTT	CGC	TGG	2654
TAC	AGA	TGG	GGT	GGA	GAA	Clu	Lou	Ara	Ara	Thr	Leu	Leu	Ara	Trp	
Tyr	Arg	Trp	GIY			GIY	neu	my	910				· ,	915	
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					a >-		225	CC	C 2 2	. אמ	. 659	CAA	ראה	ርጥጥ	2699
GCA	GCI	TCC	ATC	ACA	GAA	. AAA 	AA1	C1	GAA	, AAC	. Glu	GA.	Gla	GTT Val	
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ATT	ACT	GAA	GAT	GAA	GAA	GTT	TCT	ACG	GGT	TAT	TGC	TTT	GCG	TTT	2744
Ile	Thr	Glu	Asp	Glu	Glu	Val	Ser	Thr	Gly	Tyr	Cys	Phe	Ala	Phe	
				935					940				•	945	
										ACG					2789
Lys	Ile	Lys	Asn		Asn	Lys	Val	Pro		Thr	Lys	Glu			
				950					955				'	960	
												•			
AAG	TCA	ATG	AGG	ATT	CAA	GCT	CTT	CGT	TGC	CAT	GTG	ATT	TAC	TGT	2834
Lys	Ser	Met	Arg	Ile	Gln	Ala	Leu	Arg	Cys	His	Val	Ile	Tyr	Cys	
				965					970					975	
															·
													500	aam.	
														CGT	2879
Gln	Asn	Gly	Ser		Met	Asn	Val	TTE		Val	Leu	Ala		Arg 990	
				980					985					390	
TCT	CAA	GCC	CTC	AGG	TAT	CTT	TAT	GTT	CGT	TGG	GGA	GTT	GAG	TTG	2924
Ser	Gln	Ala	Leu	Arg	Tyr	Leu	Tyr	Val	Arg	Trp	Gly	Val	Glu	Leu	
				995]	1000				1	005	
						cm**	001	633	mem.	ccm	c) c	3.03	C 3 M	መአመ	2969
										Gly				TAT	2903
ser	гÀг	Met		vaı 1010	FIIC	VAI	Gry		1015	u Ly	nap	1		.020	
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						•									
														GGA	3014
Glu	Gly	Leu	Leu	Gly	Gly	Val	His	Lys	Thr	Val	Ile	Leu	Lys	Gly	
				1025				:	1030				1	.035	

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GTC TCC AAC ACT GCT TTA AGG TCT CTC CAT GCC AAC AGA AGT TAC	3059
Val Ser Asn Thr Ala Leu Arg Ser Leu His Ala Asn Arg Ser Tyr	
1040 1045 1050	
CCT CTT TCA CAT GTC GTG TCG CTT GAC AGC CCC AAT ATT GGC GAG	3104
Pro Leu Ser His Val Val Ser Leu Asp Ser Pro Asn Ile Gly Glu	
1055 1060 1065	
GTG AGC AAA GGG TGC AGC AGC TCC GAG ATC CAG TCC ATC GTC ACA Val Ser Lys Gly Cys Ser Ser Ser Glu Ile Gln Ser Ile Val Thr	3149
1070 1075 1080	
AAA CTC TCC AAA GCT TAA TCAGATATCT GCTGCTTTCT TTTGGGTAAG	3197
Lys Leu Ser Lys Ala End	
1085	
CAAGGTTTCA TCTTATATGA TTATATCATA AGATACTATA TAAGCACCTT	3247
Chadillon Idlinia Inches	
ATTGGTAAGT CAGTCCCATA ATAATAATGT ACTTCAGAAC CACAATACTT	3297
AAAAGTTGGT TCAGTAGTGA TTAGTCTCAT AATAATCATA TAATTACACA	3347
	2207
TCCGCTGTTA ACTAGTGGTA ATATCTAAGC TCAACAATAA AGATGTAAAA	3397
TGCTAGTATG GAAATGAATT GCTAGCTGTT GATCTCTTTC CCTTTATTCT	3447
IGGINGINIS GMAIGHEIT GOTHGOTOTT GHTGTOTTS GGTTANTA	- • • •
GTATTATTTC TTTCCTCATC TCATGTAAAA ACAATTTTCT GAAGGTGTAC	3497
AGTTTTTTCC CCTTATATAT CTGTATTATT TCTACTATTT TTTGTTTGTA	3547
	2522
AGAATATCCT CTCATCGAGG AGTGATAATT AAATAACCGG CTTGCTAAAT	3597

65

ATAAAGCTTA TTCGAGTTAA AAAAAAAAA AAAAAAAA

3635

4. DNA sequence with the coding region for sucrose-synthase for the preparation of sugar beet with modified sucrose concentration, characterised in that the sequence has the following nucleotide sequence (Seq. ID No.4):

CT GCA GGA GGG AAA CAA ATT CTT AGC GAT GGC CCG TTT AGC GAA 0044
Ala Gly Gly Lys Gln Ile Leu Ser Asp Gly Pro Phe Ser Glu
5 10

GTT CTT AGG TCT GCT CAG GAA GCA ATA GTT GTT CCT CCC TTT GTT 0089

Val Leu Arg Ser Ala Gln Glu Ala Ile Val Val Pro Pro Phe Val

15 20 25

GCT ATA GCA GTC CGT CCA AGA CCT GGA GTT TGG GAA TAT GTT CGT 0134
Ala Ile Ala Val Arg Pro Arg Pro Gly Val Trp Glu Tyr Val Arg
30 35 40

GTT AAT GTC TCT GAA CTG AAT GTG GAG CAG CTA ACT GTG TCT GAG 0179
Val Asn Val Ser Glu Leu Asn Val Glu Gln Leu Thr Val Ser Glu
45 50 55

TAT CTC CAT TTC AAG GAA GAA CTT GTG GAT GGA AAG GCT GAT GAC 0224

Tyr Leu His Phe Lys Glu Glu Leu Val Asp Gly Lys Ala Asp Asp
60 65 70

CAC TAT GTG CTT GAG CTT GAT TTC GAG CCT TTT AAT GAA TCA GTT 0269

His Tyr Val Leu Glu Leu Asp Phe Glu Pro Phe Asn Glu Ser Val

75 80 85

CCA CGT CCA ACT CGC TCT TCA TCA ATT GGT AAT GGT GTT CAG TTC 0314

Pro Arg Pro Thr Arg Ser Ser Ser Ile Gly Asn Gly Val Gln Phe
90 95 100

								66	•						
					TCA										0359
Leu	Asn	Arg	His	Leu	Ser	Ser	Ser	Met	Phe	Cys	Asn	Lys	Asp	Cys	
105					110					115					
TTG	GAG	CCG	TTA	CTT	GAT	TTT	CTT	'AGA	GTG	CAC	AAA	CAT	AAA	GGA	0404
Leu	Glu	Pro	Leu	Leu	Asp	Phe	Leu	Arg	Val	His	Lys	His	Lys	Gly	
120					125					130					
GTT	GTC	ATG	ATG	TTG	AAT	GAT	CGG	ATA	CAG	ACT	ATC	CAG	CGT	CTT	0449
					Asn										
135					140					145					
CAG	тст	GCA	TTG	TCT	AAA	GCT	GAG	GAT	TAT	CTT	ATC	AAA	CTT	CCA	0494
					Lys										
150	001				155			-		160		-			
130															
CCA	GAT	a C A	CCT	TAC	TCT	GAG	TTC	GAA	TTT	GTA	ATC	CAA	GGT	ATG	0539
					Ser										
	rah	1111	110	-1-	170		•			175			•		
165															
		63.3	3.03	ccc	TICC	CCT	САТ	ልርጥ	CCT	CAA	ACC.	ርጥጥ	ርጥል	GAA	0584
GGT	TTT	GAA	AGA	Clu	Trp	C111) cn	Whr	712	GIN	Ara	Val	T.e.ii	Glu	
	Pne	GIU	Arg	GTÅ		GIY	Asp	Till	AIG	190	nr 9	V u	ي	010	
180					185					190					
										•					
									03.5	ac=	000	6	000	m~=	0630
					CTA										0629
Met	Met	His	Leu	Leu	Leu	Asp	Ile	Leu	GIn		Pro	Asp	Pro	ser	
					~~~					205					

								0	,						
										ATG					0674
Thr	Leu	Glu	Thr	Phe		GIY	Arg	Leu		Met	val	Pne	ASI	vaī	
210					215					220					
														CTC	0719
Val	Ile	Leu	Ser	Val	His	Gly.	Tyr	Phe	Gly	Gln	Ala	His	Val	Leu	
225					230					235					
GGC	TTG	CCT	GAC	ACT	GGT	GGG	CAG	ATA	GTT	TAT	ATA	CTT	GAC	CAA	0764
Glv	Leu	Pro	Asp	Thr	Gly	Gly	Gln	Ile	Val	Tyr	Ile	Leu	Asp	Gln	
240			_		245					250					
ርጥር	cee	ጥርጥ	CTG	GAA	CAT	GAA	ATG	CTC	CAA	CGA	ATA	AAG	AAG	CAA	0809
										Arg					
	Ar 9	<i>-</i>	200		260					265		•	-		
255					200							•			
	oma.	C3 M	cmc	a cm	CCT	NG A	ያ መመ	Chuh	ልጥሮ	GTG	N.C.T.	.ccc	<b>ጥ</b> ጥG	ATT	0854
										Val					
_	Leu	Asp	vaı	Int		Ary	116	Deu	116		Der	11.9	<b></b>		
270	-				275					280					
															2000
										CGT					0899
Pro	Asp	Ala	Lys	Gly	Thr	Thr	Cys	Asn	Gln	Arg	Met	Glu	Lys	Val	
285					290					295					
									•						
						_									
														TCA	0944
Ser	Gly	Thr	Glu	His	Ala	Ser	Ile	Leu	Arg	Val	Pro	Phe	Arg	Ser	
300		•			305					310					

									•						
			ATC												0989
Glu	Lys	Gly	Ile	Leu	Arg	Lys	Trp	Ile	Ser	Arg	Phe	Asp	Val	Trp	
315			•		320					325					
								•							
CCT	TAT	TTA	GAG	ACC	TTC	ACT	GAG	GAT	GCA	GCT	GGT	GAA	ATT	ATT	1034
Pro	Tyr	Leu	Glu	Thr	Phe	Thr	Glu	Asp	Ala	Ala	Gly	Glu	Ile	Ile	
330					335					340					
GGC	GAG	TTG	CAG	GGT	CGT	CCA	GAT	CTG	ATA	ATT	GGC	AAC	TAC	AGC	1079
Gly	Glu	Leu	Gln	Gly	Arg	Pro	Asp	Leu	Ile	Ile	Gly	Asn	Tyr	Ser	
345					350					355					
														GTC	1124
Asp	Gly	Asn	Ile	Val	Ala	Ser	Leu	Leu	Ser	His	Lys	Met	Gly	Val	
360					365					370					
														CCA	1169
Thr	Gln	Cys	Asn	Ile	Ala	His	Ala	Leu	Glu	Lys	Thr	Lys	Tyr	Pro	
<b>37</b> 5					380					385					
														TTC	1214
Asp	Ser	Asp	Ile	Tyr	Trp	Lys	Arg	Phe	Glu	Asp	Lys	Tyr	His	Phe	
390					395					400					
														GAT	1259
														Ąsp	
405					410					415					

								_,	-				•		
TTC	ATC	ATT	ACG	AGT	ACT	TAC	CAA	GAG	ATA	GCT	GGA	ACG	AAG	AAT	1304
Phe	Ile	Ile	Thr	Ser	Thr	Tyr	Gln	Glu	Ile	Ala	Gly	Thr	Lys	Asn	
420					425					430					
										TTT					1349
Thr	Val	Gly	Gln	Tyr	Glu	Ser	His	Lys	Ala	Phe	Thr	Phe	Pro	Gly	
435					440					445					
										TTT					1394
Leu	Tyr	Arg	Val	Val	His	Gly	Ile	Asp	Val	Phe	Asp	Pro	Lys	Phe	
450					455					460					
									•						
														mann	1 4 2 0
										ATC					1439
Asn	Ile	Val	Ser	Pro		Ala	Asp	Met	Ala	Ile	Tyr	Phe	Pro	Pne	
465					470					475					
							cm.c	<b>3</b> cm	<b>m</b> ax	comm.	C > m		C TOTAL	N/III N	7404
										CTT					1484
	Glu	Lys	Asp	Val		Cys	Leu	Thr	ser	Leu	HIS	Arg	Leu	TIG	
480					485					490					
											•				
			<b>-</b>	mma		COTI	CAC	CAC	እአሮ		CAA	CAC	לואנו ע	CCT	1529
										GAA					1327
	GIN	Leu	Leu	Pne		PIU	GIU	GIII	KSII	Glu 505		mra	116	GLY	
495					500					303	•				
						•									
~m>	നസ	ሮአጥ	CAT	እርር	ጥሮኔ	AAG	CCA	<b>ልጥጥ</b>	АТА	TTT	TCC	ATG	GCG	AGG	1574
										Phe					•
	ren	wsh	rop		515	-1-				520					
510															

								70	)						
	<b>63.6</b>	acm	GTG	a a c	ልልጥ	מידמ	<b>DCD</b>	GGG	CTG	СТА	GAG	TGC	тат	GGC	1619
			Val												
	Asp	Arg	Val	гåз	530	116	1111	GIY		535	014	0,10	-1-	1	
525					330					<i></i>					
			AAA	0.TT.C	3.00	C33	CMC.	.ccv	220	CTG.	CTT	СПЗ	GTG	GCT	1664
			AAA Lys												2001
_	Asn	ATS	Lys	Leu		GIU	Ded	Ald		550°	vai	***	•••	****	
540					545					330					
		•							a. a		63.6	C 3 3	እመጥ	ccc	1709
														GCC	1703
Gly	Tyr	Asn	Asp	Val		Lys	ser	Asn	Asp		GIU.	GIU	ITE	ALG	
555					560					565					
															1764
														AGA	1754
Glu	Ile	Glu	Ĺys	Met		Arg	Leu	Ile	Gln		Tyr	ASN	Leu	Arg	
570					575					580					
														AAT	1799
Gly	Gln	Phe	Arg	Trp	Ile	Ala	Ser	Gln	Thr		Arg	Val	Arg	.Asn	
585					590					595					
														GCG	1844
Gly	Glu	Leu	Tyr	Arg	Tyr	Ile	Cys	Asp	Lys	Gly	Gly	Ile	Phe	Ala	
600					605				•	610					
						_									
						•									
														GCC	1889
Glr	Pro	Ala	Phe	Tyr	G۱u	Ala	Phe	Gly	Leu	Thr	Val	Val	Glu	Ala	
615	i				620					625					

									•						
											CAC				1934
Met	Thr	Cys	Gly	Leu	Pro	Thr	Phe	Ala	Thr	Cys	His	Gly	Gly	Pro	
630					635					640					
														•	
													~~ m	003	1070
											CAT				1979
Ala	Glu	Ile	Ile	Glu		Gly	Val	Ser			HIS	116	ASP	Pro	•
645					650					655					
														-	
<b>~</b> .~	<b>~</b> \ <b>m</b>	com	_C አ ጦ	CAG	GCA	CAA	AAA	ATG	ACT	GAA	TTC	TTT	GTC	AAG	2024
											Phe				
660	UIS	NIG	лор	<b>U</b> 111	665	-	-2-			670				_	
860												٠			
												,			
TGC	AGA	GAG	GAT	CCA	AAC	TAC	TGG	ACT	AAA	ATC	TCT	GCA	GGA	GGG	2069
											Ser				
675	•				680					685					
														GAA	2114
Leu	Leu	Arg	Ile	Lys	Glu	Arg	Tyr	Thr	Trp		Lys	Tyr	Ser	Glu	
690					695					700					
											mee		m s m	CMC	2159
AGG	TTA	ATG	ACA	TTG	GCA	GGG	GTG	TAT	GGT	TIC	TGG	AAA	TAL	GTC	. 2133
Arg	Leu	Met	Thr	Leu		GIY	Val	туг	GIY		ııp		TÄT	Val	
705	•	•			710					715					
<b></b>		OM3	CNC	י אכא	אמא	GAG	ACA	CGA	CGT	TAT	CTT	GAG	ATG	TTC	2204
TCT	AAA	CIA	י הווי	Arn	Ard	Glu	Thr	Ara	Arq	Tyr	Leu	Glu	Met	Phe	
		, Let	GIU	nry	725			7		730					
720															

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72

														GCA	2249
ryr	Ile	Leu	Lys	Phe	Arg	Asp	Leu	Ala	Asn	Ser	Val	Pro	Leu	Ala	
735					740					745					
•												•			
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ACA	GAT	GAA	GAG	CCT	TCT	ACT	ACT	GAT	GCA	GTT	GCG	ACA	TTC	CGT	2294
Thr	Asp	Glu	Glu	Pro		Thr	Thr	Asp			Ala	Thr	Pne	Arg	
750					<b>75</b> 5					760					
					na'a 1	700 3 C	тс <b>х с</b> (	~m m/	מרא א	رعامات	יי כייי	አጥስጥ	ልጥጥል		2343
			ACG	CTGC.	rge :	PTAC.	IGAG	31 10	CAA	GIIG.	1 61	nini.	n. in		2545
Gly	Pro	End													
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CTG.	CMM	466	anin	ngio.											
المس	<b>سارىلىر</b>	TG	TAAA'	TAAC	GA G.	AGTG.	AAAA	A TG	TAAT	ATTG	TTG	ATGT	CTT		2443
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GAAZ	AACT	GAG	TTTG	CTTT	GT T	TATT	TTTA	A GT	GTÀT	GACA	ATA	TGTA	TCA		2493
TAT	AACG	GAT	TCTT	CAGT	GA T	CATA	TCAA	A AA	CTAC	TGAC	CAT	CGAA	GTT		2543
														•	
AAT	GAAA	ATC	GACA	GCAA	CA										2563
	_		<b>~</b>			af Di	MA e	ומוואם	nces	200	ordi	na t	o an	v one	of

- 5. Derivatives of DNA sequences according to any one of claims 1 to 4 characterised in that these derivatives are obtained by exchange of single bases or by targeted or non-targeted mutagenesis.
- 6. Plasmids containing

5

a) a suitable promoter which ensures that the coding sequence meets a suitable time point or in the specified developments in the transgenic plant or in determined genes of transgenic plants;

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- at least one coding sequence according to b) claims 1 to 5, that is so coupled to the promoter that the i) formation of an RNA is allowed which is into a protein, whereby the protein 5 demonstrates an enzymatic activity which leads to a modification of the sucrose concentration in the plant, or which is so coupled to the promoter that ii) the coding part is read, which leads to 10 the formation of a so-called anti-sense RNA which under-expresses the formation of the protein coded from an endogenous gene in the plant, that is involved in the sucrose biosynthesis; and 15
  - c) A non-coding termination sequence that contains the signal for the termination and polyadenylation of the transcript.
- Use of the DNA-sequences according to any one of claims 1 to 6 for the preparation of sugar beet with changed sucrose concentration.
- Use of the plasmids according to any one of claims 1-4 for the preparation of derivatives by targeted or non-targeted mutagenesis
- 9. Sugar beet containing a DNA sequence according to any one of claims 1-5.

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